

## TENT COOPERATION TRE Y

PCT

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
 United States Patent and Trademark  
 Office  
 Box PCT  
 Washington, D.C.20231  
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

|                                  |
|----------------------------------|
| Date of mailing (day/month/year) |
| 07 January 2000 (07.01.00)       |

|  |                                       |
|--|---------------------------------------|
| International application No.              | Applicant's or agent's file reference |
| PCT/EP99/03603                             | FB/BM45323                            |
| International filing date (day/month/year) | Priority date (day/month/year)        |
| 26 May 1999 (26.05.99)                     | 26 May 1998 (26.05.98)                |

|                    |
|--------------------|
| Applicant          |
| RUELLE, Jean-Louis |

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:  
 \_\_\_\_\_  
 03 December 1999 (03.12.99)

in a notice effecting later election filed with the International Bureau on:  
 \_\_\_\_\_

2. The election  was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

|   |   |
|---|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br>A. Karkachi<br>Telephone No.: (41-22) 338.83.38 |
|---|---|



## TENT COOPERATION TRE

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

|  |
|--|
| Date of mailing (day/month/year)<br>18 January 2000 (18.01.00) |
|--|

|   |
|---|
| To:<br><br>TYRRELL, Arthur, William, Russell<br>SmithKline Beecham<br>Two New Horizons Court<br>Brentford<br>Middlesex TW8 9EP<br>ROYAUME-UNI |
|---|

|   |  |
|---|--|
| Applicant's or agent's file reference<br>FB/BM45323 | <b>IMPORTANT NOTIFICATION</b>  |
| International application No.<br>PCT/EP99/03603     | International filing date (day/month/year)<br>26 May 1999 (26.05.99) |

|  |                      |                    |
|--|----------------------|--------------------|
| 1. The following indications appeared on record concerning:  |                      |                    |
| <input checked="" type="checkbox"/> the applicant <input checked="" type="checkbox"/> the inventor <input type="checkbox"/> the agent <input type="checkbox"/> the common representative |                      |                    |
| Name and Address   | State of Nationality | State of Residence |
|  |                      |                    |
|  | Telephone No.        |                    |
|  | Facsimile No.        |                    |
|  | Teleprinter No.      |                    |

|   |                            |                          |
|---|----------------------------|--------------------------|
| 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:   |                            |                          |
| <input checked="" type="checkbox"/> the person <input checked="" type="checkbox"/> the name <input checked="" type="checkbox"/> the address <input checked="" type="checkbox"/> the nationality <input checked="" type="checkbox"/> the residence |                            |                          |
| Name and Address<br>TOMMASSEN, Johannes, Petrus, Maria<br>Utrecht University<br>Dept. of Molecular Cell Biology<br>Padualaan 8<br>NL-3584 CH Utrecht<br>Netherlands   | State of Nationality<br>NL | State of Residence<br>NL |
|   | Telephone No.              |                          |
|   | Facsimile No.              |                          |
|   | Teleprinter No.            |                          |

|  |
|--|
| 3. Further observations, if necessary:<br><b>Applicant/inventor for the United States of America has been added. A power of attorney is needed for TOMMASSEN, Johannes, Petrus, Maria.</b> |
|--|

|  |
|--|
| 4. A copy of this notification has been sent to:   |
| <input checked="" type="checkbox"/> the receiving Office <input type="checkbox"/> the designated Offices concerned               |
| <input type="checkbox"/> the International Searching Authority <input checked="" type="checkbox"/> the elected Offices concerned |
| <input checked="" type="checkbox"/> the International Preliminary Examining Authority <input type="checkbox"/> other:            |

|   |   |
|---|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br><br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br><br>Athina Nickitas-Etienne<br><br>Telephone No.: (41-22) 338.83.38 |
|---|---|




**PATENT COOPERATION TREATY**
**PCT**
**NOTIFICATION OF THE RECORDING  
OF A CHANGE**

(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

|                                  |
|----------------------------------|
| Date of mailing (day/month/year) |
| 18 January 2000 (18.01.00)       |

From the INTERNATIONAL BUREAU

To:

TYRRELL, Arthur, William, Russell  
SmithKline Beecham  
Two New Horizons Court  
Brentford  
Middlesex TW8 9EP  
ROYAUME-UNI

|   |  |
|---|--|
| Applicant's or agent's file reference<br>FB/BM45323 | <b>IMPORTANT NOTIFICATION</b>  |
| International application No.<br>PCT/EP99/03603     | International filing date (day/month/year)<br>26 May 1999 (26.05.99) |

|   |  |                                    |  |  |
|---|--|------------------------------------|--|--|
| 1. The following indications appeared on record concerning: |  |                                    |  |  |
| <input checked="" type="checkbox"/> the applicant           | <input checked="" type="checkbox"/> the inventor | <input type="checkbox"/> the agent | <input type="checkbox"/> the common representative |  |
| Name and Address  | State of Nationality                             |                                    | State of Residence                                 |  |
|   |  |                                    |  |  |
|   | Telephone No.                                    |                                    |  |  |
|   | Facsimile No.                                    |                                    |  |  |
| Teleprinter No.   |  |                                    |  |  |

|   |  |   |   |   |
|---|--|---|---|---|
| 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:   |  |   |   |   |
| <input checked="" type="checkbox"/> the person  | <input checked="" type="checkbox"/> the name | <input checked="" type="checkbox"/> the address | <input checked="" type="checkbox"/> the nationality | <input checked="" type="checkbox"/> the residence |
| Name and Address<br><br>TOMMASSEN, Johannes, Petrus, Maria<br>Utrecht University<br>Dept. of Molecular Cell Biology<br>Padualaan 8<br>NL-3584 CH Utrecht<br>Netherlands | State of Nationality                         |   | State of Residence                                  |   |
|   | NL   |   | NL  |   |
|   | Telephone No.                                |   |   |   |
|   | Facsimile No.                                |   |   |   |
| Teleprinter No.   |  |   |   |   |

|  |  |  |   |  |
|--|--|--|---|--|
| 3. Further observations, if necessary:<br><b>Applicant/inventor for the United States of America has been added. A power of attorney is needed for TOMMASSEN, Johannes, Petrus, Maria.</b> |  |  |   |  |
| 4. A copy of this notification has been sent to:   |  |  |   |  |
| <input checked="" type="checkbox"/> the receiving Office   |  |  | <input type="checkbox"/> the designated Offices concerned         |  |
| <input type="checkbox"/> the International Searching Authority   |  |  | <input checked="" type="checkbox"/> the elected Offices concerned |  |
| <input checked="" type="checkbox"/> the International Preliminary Examining Authority  |  |  | <input type="checkbox"/> other:                                   |  |

|   |   |
|---|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland | Authorized officer<br><br>Athina Nickitas-Etienne |
| Facsimile No.: (41-22) 740.14.35  | Telephone No.: (41-22) 338.83.38                  |



**PATENT COOPERATION TREATY**  
**PCT**

**INTERNATIONAL SEARCH REPORT**

(PCT Article 18 and Rules 43 and 44)

|   |   |   |
|---|---|---|
| Applicant's or agent's file reference<br><br>FB/BM45323 | <b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |   |
| International application No.<br><br>PCT/EP 99/03603    | International filing date (day/month/year)<br><br>26/05/1999  | (Earliest) Priority Date (day/month/year)<br><br>26/05/1998 |
| Applicant<br><br>SMITHKLINE BEECHAM BIOLOGICALS S.A.    |   |   |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
    - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
  - b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
    - contained in the international application in written form.
    - filed together with the international application in computer readable form.
    - furnished subsequently to this Authority in written form.
    - furnished subsequently to this Authority in computer readable form.
    - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
    - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
2.  **Certain claims were found unsearchable** (See Box I).
3.  **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

**NIESSERIA MENINGITIDIS ANTIGENIC POLYPEPTIDES, CORRESPONDING POLYNUCLEOTIDES AND PROTECTIVE ANTIBODIES**

5. With regard to the **abstract**,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

8

None of the figures.



1

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/EP 99/03603

**Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)**

The invention provides *neisseria meningitidis* BASB030 polypeptides and polynucleotides encoding BASB030 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are antibodies, diagnostic, prophylactic and therapeutic uses thereof.



## INTERNATIONAL SEARCH REPORT

International Application No

/EP 99/03603

## A. CLASSIFICATION OF SUBJECT MATTER

|       |            |           |            |           |           |
|-------|------------|-----------|------------|-----------|-----------|
| IPC 6 | C12N15/31  | C12N1/21  | C07K14/22  | C07K16/12 | G01N33/53 |
|       | G01N33/569 | A61K31/70 | A61K39/095 | A61K39/40 | A61K48/00 |

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| X          | <p>DATABASE EMPRO1<br/> E.M.B.L. Databases<br/> Accession Number: U40596,<br/> 3 February 1996 (1996-02-03)<br/> DRAKE S ET AL: "Neisseria gonorrhoeae<br/> pilus biogenesis gene cluster, pilO, pilP<br/> and pilQ genes, complete cds"<br/> XP002124749<br/> 96.5% identity in 2314 nt overlap with<br/> SeqIdNo.1 / 86.9% identity in 758 aa<br/> overlap with SeqIdNo.2<br/> abstract</p> <p>-&amp; DRAKE S ET AL : "The product of the<br/> pilQ gene is essential for the biogenesis<br/> of type IV pili in Neisseria gonorrhoeae"<br/> MOL. MICROBIOL.,<br/> vol. 18, no. 5, December 1995 (1995-12),<br/> pages 975-986, XP000857454<br/> figure 2</p> | 1,7-10,<br>17-20,24   |
| X          |   | 1,7-10,<br>17-20,24   |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## ° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

|  |  |
|--|--|
| Date of the actual completion of the international search  | Date of mailing of the international search report |
| 22 December 1999   | 11/01/2000   |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Authorized officer<br><br>Lonnoy, O                |



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/03603

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|----------|--|-----------------------|
| P, X     | <p>-----</p> <p>DATABASE EMPR01<br/>E.M.B.L. Databases<br/>Accession Number: AF066056,<br/>18 December 1998 (1998-12-18)<br/>TONJUM T ET AL: "Neisseria meningitidis<br/>secretin precursor (pilQ) gene, complete<br/>cds"<br/>XP002124750<br/>97.9% identity in 2339 bp overlap with<br/>SeqIdNo.1 / 85.6% identity in 766 aa<br/>overlap with SeqIdNo.2<br/>abstract</p> | 1,7-10,<br>17-20,24   |
| P, X     | <p>-&amp; TONJUM T ET AL: "Structure and<br/>function of repetitive sequence elements<br/>associated with a highly polymorphic<br/>domain of the Neisseria meningitidis PilQ<br/>protein"<br/>MOL. MICROBIOL.,<br/>vol. 29, no. 1, July 1998 (1998-07), pages<br/>111-124, XP000863074<br/>figure 1B</p> <p>-----</p>  | 1,7-10,<br>17-20,24   |
| A        | <p>WO 98 02547 A (INST NAT SANTE RECH MED<br/>;MAX PLANCK GESELLSCHAFT (DE); SMITHKLINE)<br/>22 January 1998 (1998-01-22)</p> <p>-----</p>   | 1-28                  |
| A        | <p>EP 0 301 992 A (NACIONAL DE BIOPREPARADOS<br/>CENT) 1 February 1989 (1989-02-01)</p> <p>-----</p>   | 1-28                  |
| A        | <p>US 4 271 147 A (HELTING TORSTEN B ET AL)<br/>2 June 1981 (1981-06-02)</p> <p>-----</p>  | 1-28                  |
| A        | <p>PETTERSSON A ET AL: "Monoclonal<br/>antibodies against the 70-kilodalton<br/>iron-regulated protein of Neisseria<br/>meningitidis are bactericidal and strain<br/>specific"<br/>INFECT. IMMUN.,<br/>vol. 58, no. 9, September 1990 (1990-09),<br/>pages 3036-3041, XP002124748</p> <p>-----</p>   | 1-28                  |



## INTERNATIONAL SEARCH REPORT

Information on patent family members

National Application No

/EP 99/03603

| Patent document cited in search report | Publication date |            | Patent family member(s)  | Publication date   |
|--|------------------|------------|--|--|
| WO 9802547                             | A                | 22-01-1998 | FR 2751000 A<br>AU 3697797 A<br>EP 0951552 A   | 16-01-1998<br>09-02-1998<br>27-10-1999   |
| EP 0301992                             | A                | 01-02-1989 | AT 122893 T<br>AU 615461 B<br>AU 2031288 A<br>AU 5319794 A<br>AU 706213 B<br>AU 7422696 A<br>AU 8134991 A<br>DE 3853854 D<br>DE 3853854 T<br>ES 2074445 T<br>GR 3017218 T<br>IN 167607 A<br>JP 1125328 A<br>RU 2023448 C<br>US 5597572 A<br>US 5747653 A | 15-06-1995<br>03-10-1991<br>25-05-1989<br>24-03-1994<br>10-06-1999<br>20-02-1997<br>31-10-1991<br>29-06-1995<br>08-02-1996<br>16-09-1995<br>30-11-1995<br>24-11-1990<br>17-05-1989<br>30-11-1994<br>28-01-1997<br>05-05-1998 |
| US 4271147                             | A                | 02-06-1981 | NONE   |  |



**CASE NO: BM45323**

**PCT APPLN. NO.: PCT/EP99/03603**

**Citations enclosed with search report mailed 11 January 2000**





## PATENT COOPERATION TREATY

RECEIVED

24 AUG 2000

NEW HORIZONS COURT

PCT

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Tyrrell, Arthur R.  
SMITHKLINE BEECHAM PLC  
Corporate Intellectual Property  
Two New Horizons Court  
Brentford  
Middlesex TW8 9EP  
GRANDE BRETAGNE

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing  
(day/month/year)

18.08.00

Applicant's or agent's file reference  
FB/SH/45323

## IMPORTANT NOTIFICATION

International application No.  
PCT/EP99/03603

International filing date (day/month/year)  
26/05/1999

Priority date (day/month/year)  
26/05/1998

Applicant  
SMITHKLINE BEECHAM BIOLOGICALS S.A.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

## 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

European Patent Office  
D-80298 Munich  
Tel. +49 89 2399 - 0 Tx: 523656 epmu d  
Fax: +49 89 2399 - 4465

Authorized officer

Vullo, C

Tel. +49 89 2399-8061





## PATENT COOPERATION TREATY

PCT

REC'D 23 AUG 2000

WIPO

PCT

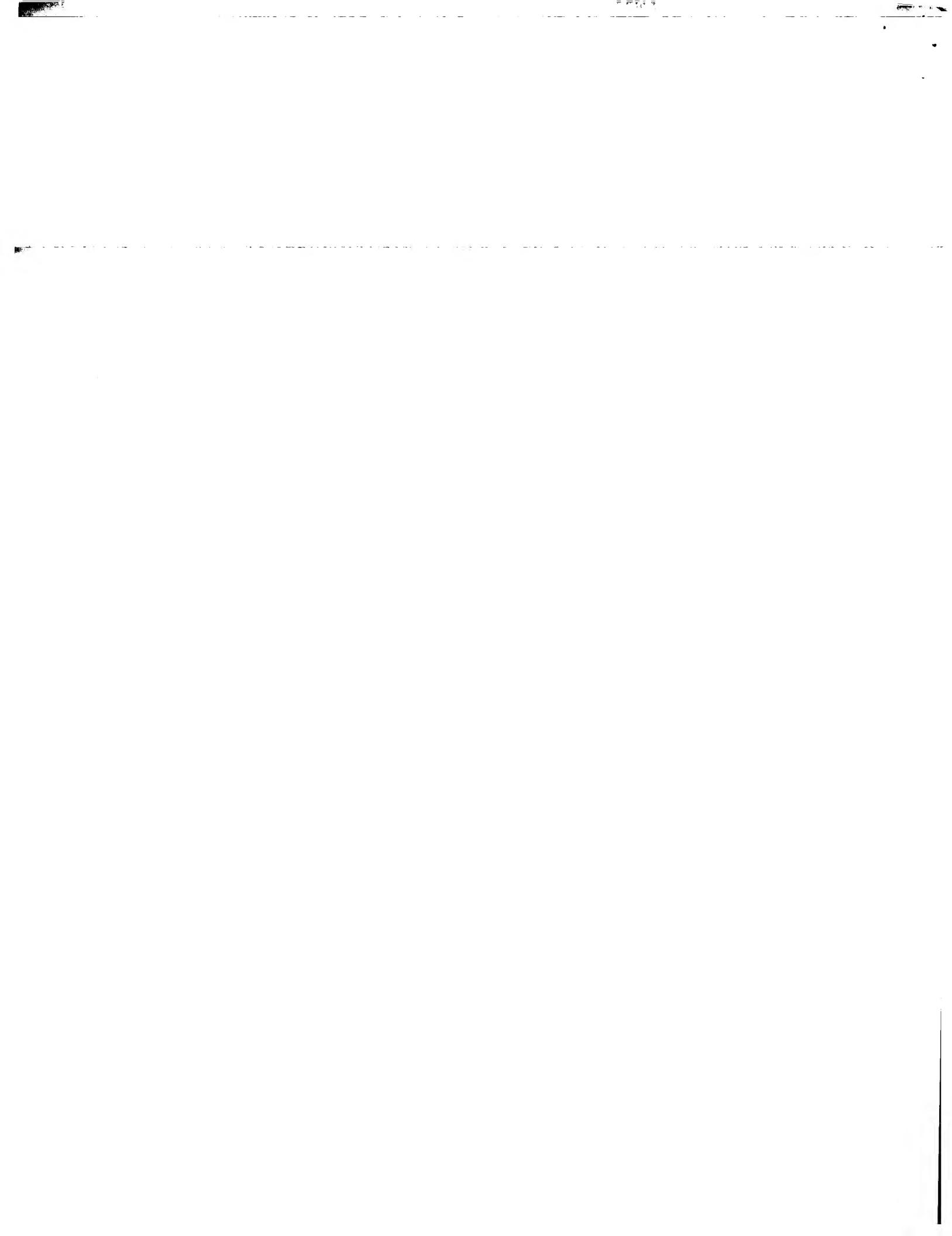
## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

|  |  |  |   |
|--|--|--|---|
| Applicant's or agent's file reference<br>FB/SH/45323   | <b>FOR FURTHER ACTION</b>                                |  | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
| International application No.<br>PCT/EP99/03603  | International filing date (day/month/year)<br>26/05/1999 | Priority date (day/month/year)<br>26/05/1998 |   |
| International Patent Classification (IPC) or national classification and IPC<br>C12N15/31  |  |  |   |
| <p>Applicant<br/>SMITHKLINE BEECHAM BIOLOGICALS S.A.</p> <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 4 sheets.</p> <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <li>I   <input checked="" type="checkbox"/> Basis of the report</li> <li>II   <input type="checkbox"/> Priority</li> <li>III   <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li>IV   <input type="checkbox"/> Lack of unity of invention</li> <li>V   <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li>VI   <input type="checkbox"/> Certain documents cited</li> <li>VII   <input type="checkbox"/> Certain defects in the international application</li> <li>VIII   <input type="checkbox"/> Certain observations on the international application</li> </ul> |  |  |   |

|  |  |
|--|--|
| Date of submission of the demand<br>03/12/1999   | Date of completion of this report<br>18.08.00                        |
| Name and mailing address of the international preliminary examining authority:<br><br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Grosskopf, R<br>Telephone No. +49 89 2399 8714 |





**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP99/03603

**I. Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-61                   as originally filed

**Claims, No.:**

1-30                   as received on                   10/08/2000   with letter of                   10/08/2000

**Drawings, sheets:**

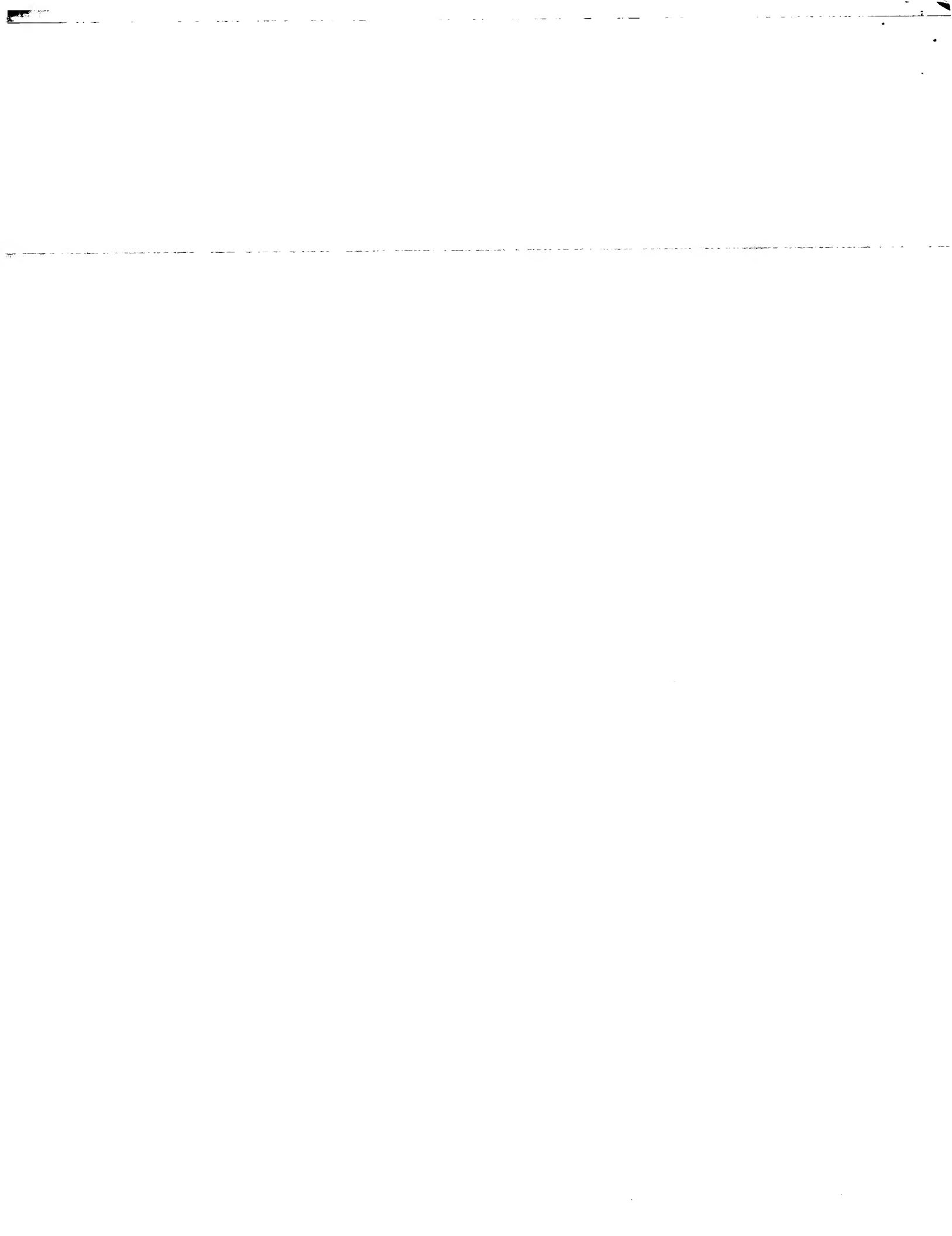
1/17-17/17           as originally filed

2. The amendments have resulted in the cancellation of:

- the description,       pages:  
 the claims,           Nos.:  
 the drawings,         sheets:

3.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP99/03603

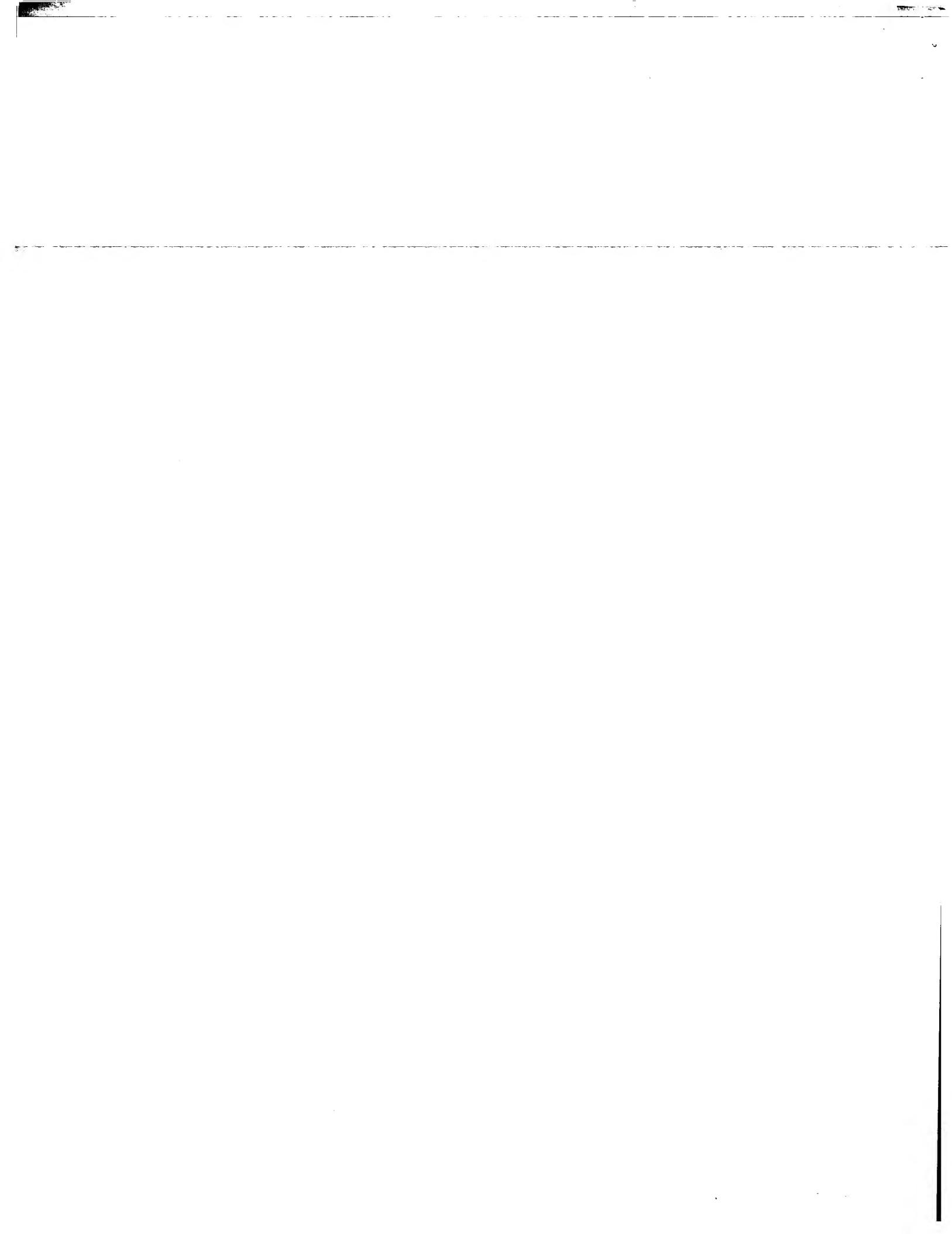
**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

|                               |      |             |
|-------------------------------|------|-------------|
| Novelty (N)                   | Yes: | Claims 1-30 |
|                               | No:  | Claims      |
| Inventive step (IS)           | Yes: | Claims      |
|                               | No:  | Claims 1-30 |
| Industrial applicability (IA) | Yes: | Claims 1-30 |
|                               | No:  | Claims      |

**2. Citations and explanations**

**see separate sheet**



INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/EP99/03603

**Ad item V:**

The Applicant has isolated a nucleotide sequence from *Neisseria meningitidis* which was arbitrarily designated "BASB030".

Although a function has not been determined, the corresponding protein seems to be immunogenic and anti-BASB030 antibodies seem to be present in human convalescent patients.

As far as the complete length proteins and polynucleotides are concerned, novelty has to be acknowledged.

On the other hand, the protein and the corresponding DNA sequence show a very high degree of identity to the pilQ gene (product) (96.5% on the nucleotide level and 86.2% on the amino acid level (see D1; DATABASE EMPRO1 E.M.B.L.

Databases Accession Number: U40596, 3 February 1996 (1996-02-03) DRAKE S ET AL: "Neisseria gonorrhoeae pilus biogenesis gene cluster, pilO, pilP and pilQ genes, complete CDS" XP002124749 -& DRAKE S ET AL : "The product of the pilQ gene is essential for the biogenesis of type IV pili in *Neisseria gonorrhoeae*" MOL. MICROBIOL., vol. 18, no. 5, December 1995 (1995-12), pages 975-986, XP000857454 ).

Thus, immunogenic fragments are not distinguishable from immunogenic fragments of the polypeptide of D1. The same applies for antibodies which are directed to the proteins

Therefore, Claims 6 and 26 at least lack an inventive activity.

But even claims which are directed to the specific proteins and/or polynucleotides and claims which are dependent thereon, although novel, seem to lack an inventive activity.

In fact, it is known that *Neisseria gonorrhoeae* and *Neisseria meningitidis* are closely related (see D2 which although published after the priority date may be cited as an "expert opinion" relating to the common knowledge; see introduction of D2).

Thus, a skilled person is aware of the probability of the existence of a



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corresponding gene in both organisms and it appears to be obvious to isolate the protein corresponding to pilQ from *Neisseria meningitidis* and to use it for the same purposes as described in D1.

Another way to isolate or identify a corresponding protein or DNA is the screening of databases which, with all due respect, does not require any inventive activity (the method used by the Applicant).

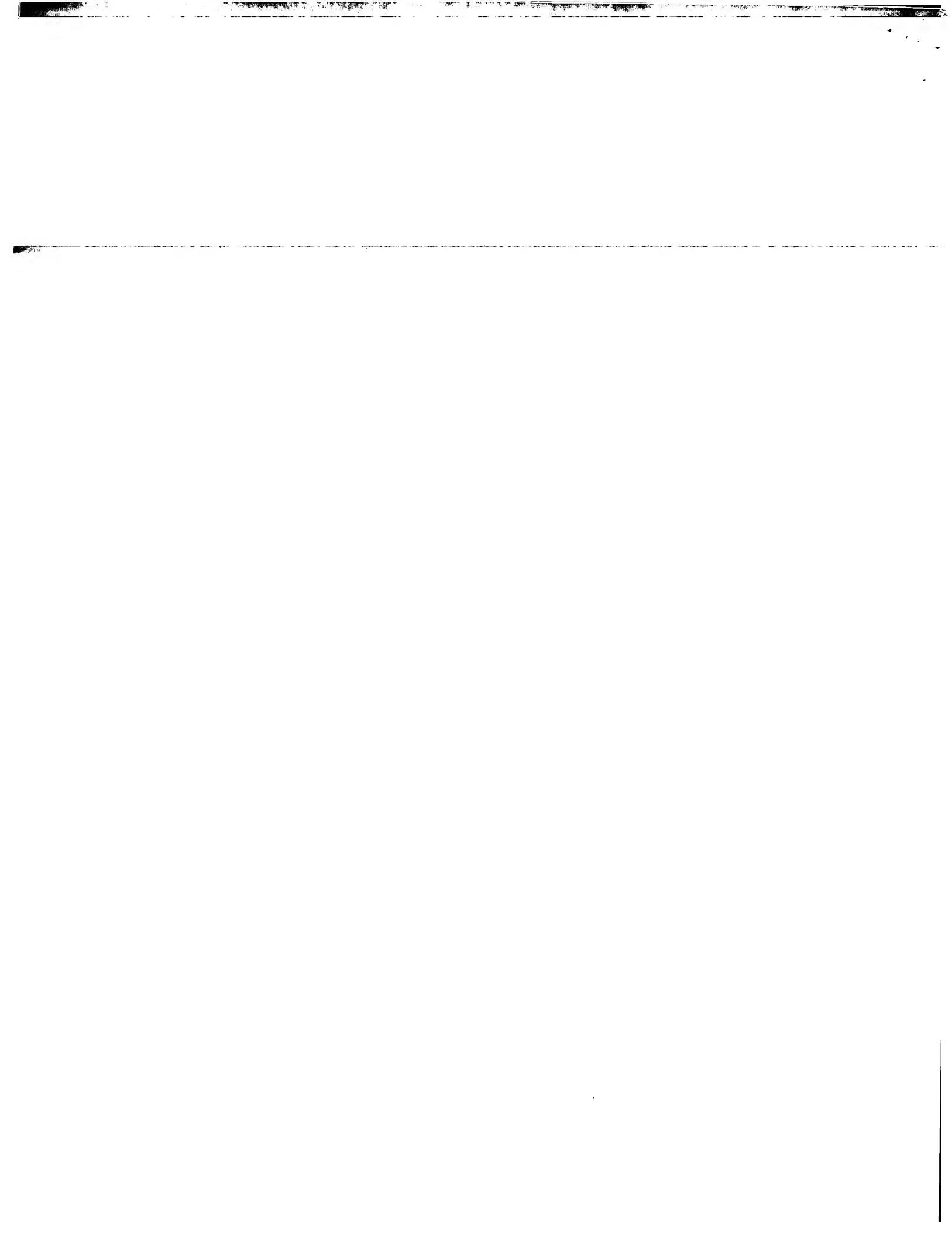
If at all, an inventive activity could, at best be established by a surprising or advantageous effect of the protein. Since, however, not even the function has been determined the demonstration of such an effect must fail.

Even if the "BASB030" should not be the same protein (which cannot be decided in the absence of the determination of any function), it would have been necessarily found when using the pilQ as a probe.

Therefore, none of the claims fulfils the requirements of Article 33.3 PCT.

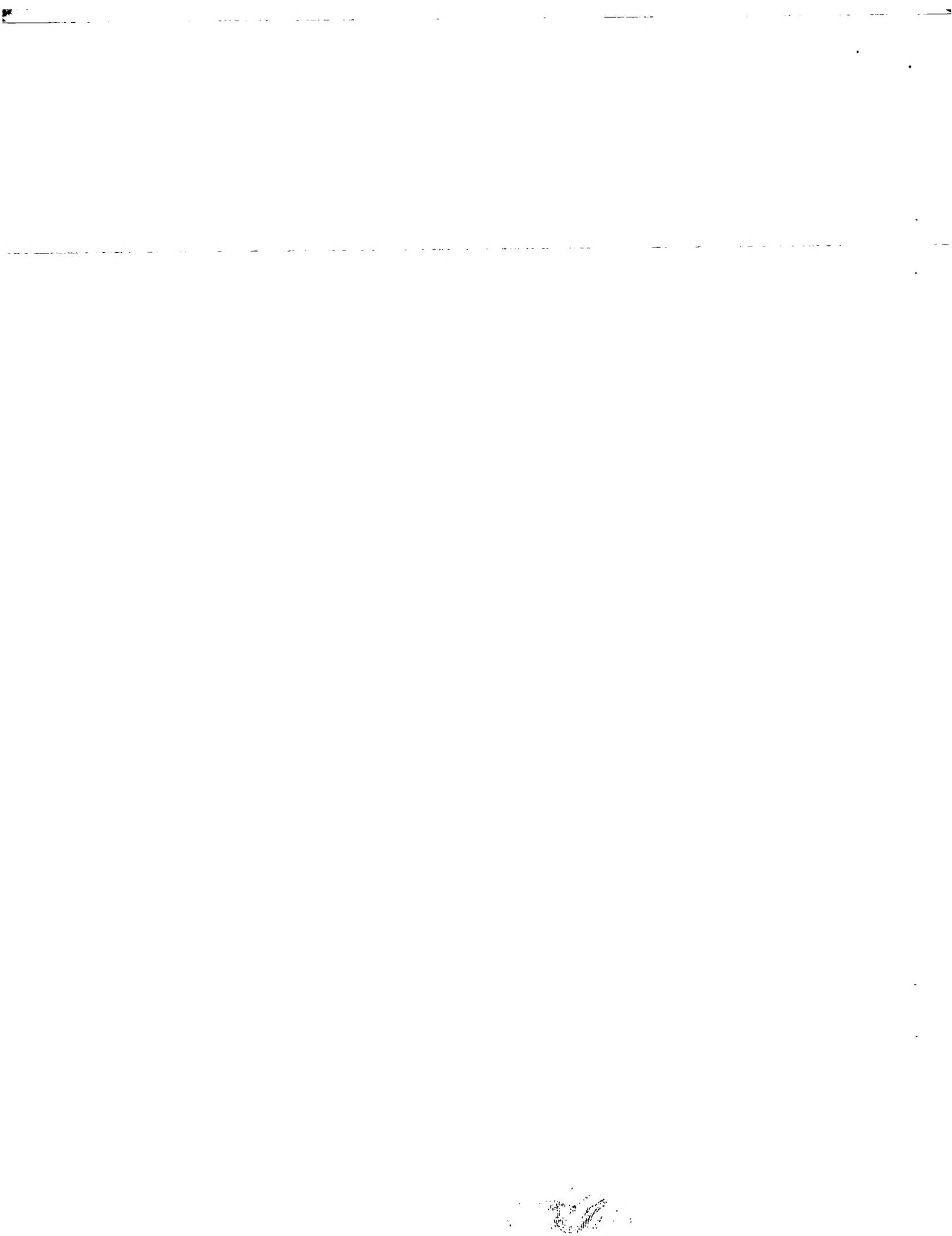
The same also applies for the dependent claims which relate to the polypeptides or polynucleotides (i.e. the vectors, host cells and processes).

Moreover, claims which are directed to vaccines and therapeutic compositions also lack at least an inventive activity, since the use of the pilQ as a vaccine is proposed in D1.



**CLAIMS:**

1. An isolated polypeptide comprising an amino acid sequence which has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:6.  
5
2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:6.  
10
3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:6.  
15
4. An isolated polypeptide of SEQ ID NO:4 and SEQ ID NO:6.  
15
5. An isolated polypeptide of SEQ ID NO:2.  
20
6. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 5 in which the immunogenic activity of said immunogenic fragment is substantially the same as the polypeptide of SEQ ID NO:4 or SEQ ID NO:6.  
25
7. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 85% identity to the amino acid sequence of SEQ ID NO: 4 or 6 over the entire length of SEQ ID NO: 4 or 6 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.  
25
8. An isolated polynucleotide comprising a nucleotide sequence that has at least 85% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO: 4 or 6 over the



entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

9. An isolated polynucleotide which comprises a nucleotide sequence which has at least 5 85% identity to that of SEQ ID NO: 3 or 5 over the entire length of SEQ ID NO: 3 or 5 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

10. The isolated polynucleotide as claimed in any one of claims 7 to 9 in which the identity is at least 95% to SEQ ID NO: 3 or 5.

10

11. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:4 or SEQ ID NO:6.

12. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:3 or SEQ 15 ID NO:5.

13. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:4 or SEQ ID NO:6, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID 20 NO:3 or SEQ ID NO:5 or a fragment thereof.

14. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2.

25 15. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1.

16. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, obtainable by screening an appropriate library under stringent



hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof.

17. An expression vector or a recombinant live microorganism comprising an isolated  
5 polynucleotide according to any one of claims 7 - 16.

18. A host cell comprising the expression vector of claim 17 or a subcellular fraction or a  
membrane of said host cell expressing an isolated polypeptide comprising an amino acid  
sequence that has at least 85% identity to the amino acid sequence selected from the group  
10 consisting of: SEQ ID NO:4 or SEQ ID NO:6.

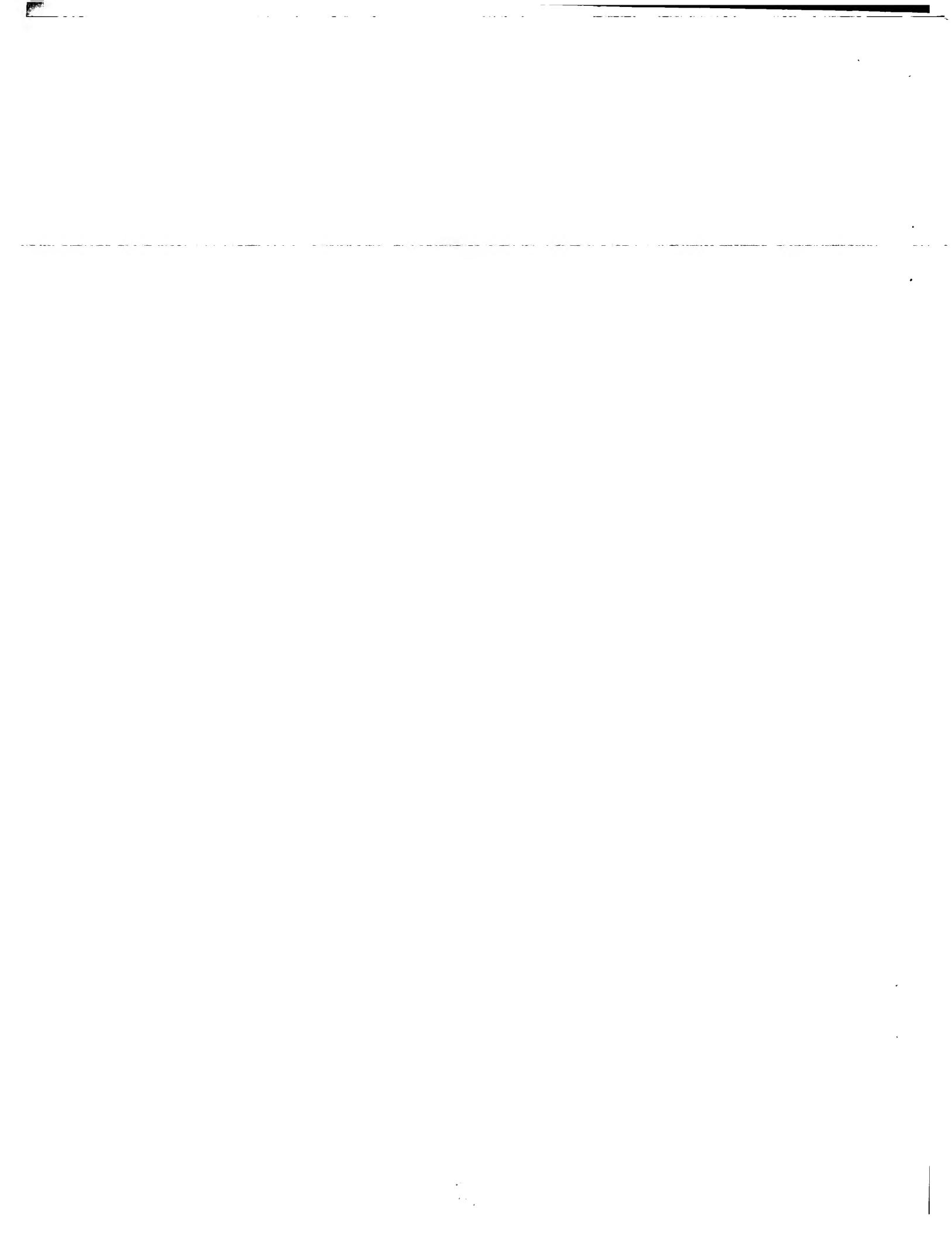
19. A process for producing a polypeptide comprising an amino acid sequence that has at  
least 85% identity to the amino acid sequence selected from the group consisting of SEQ  
ID NO:4 or SEQ ID NO:6 comprising culturing a host cell of claim 18 under conditions  
15 sufficient for the production of said polypeptide and recovering the polypeptide from the  
culture medium.

20. A process for expressing a polynucleotide of any one of claims 7 – 16 comprising  
transforming a host cell with the expression vector comprising at least one of said  
20 polynucleotides and culturing said host cell under conditions sufficient for expression of  
any one of said polynucleotides.

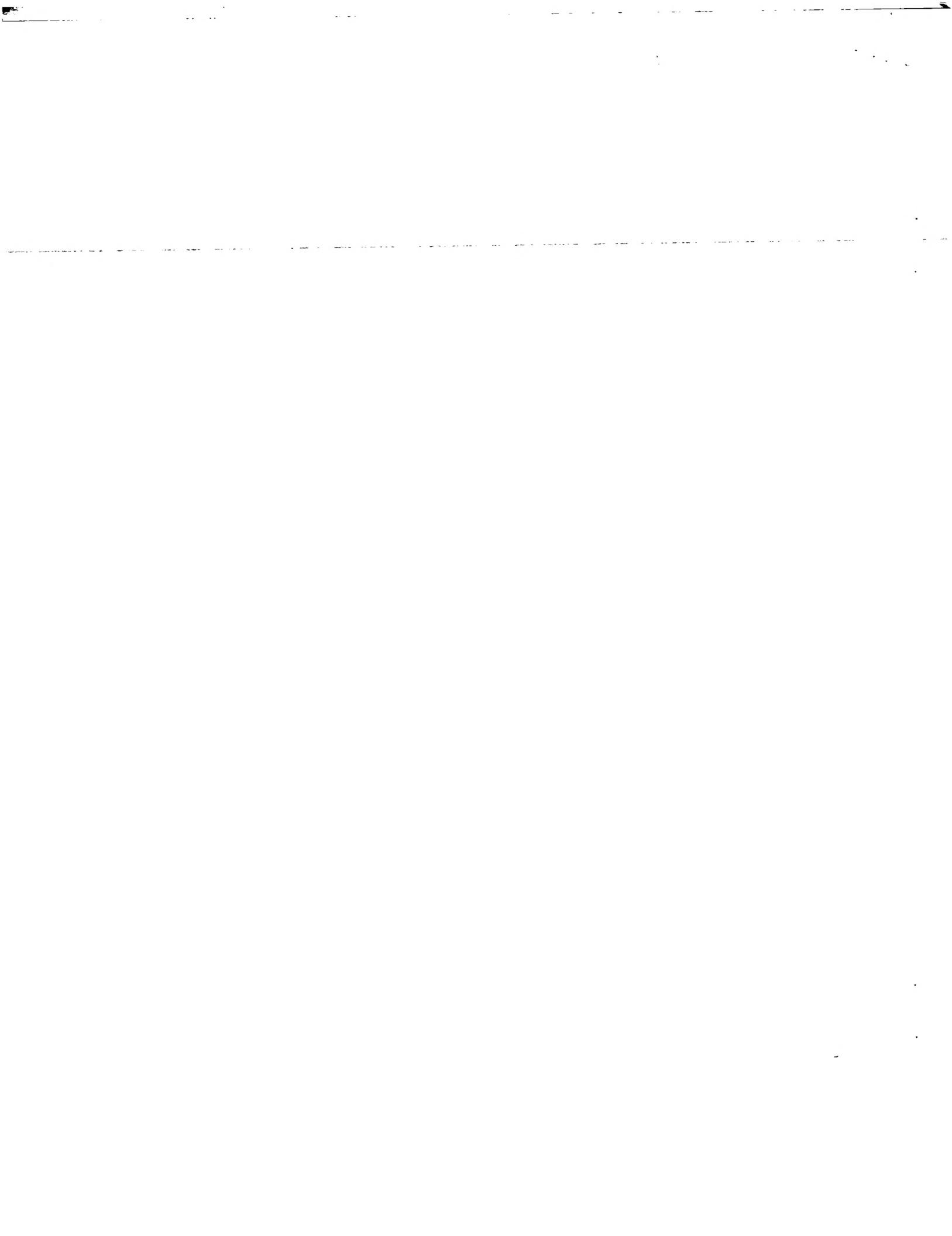
21. A vaccine composition comprising an effective amount of the polypeptide of any  
one of claims 1 to 6 and a pharmaceutically acceptable carrier.

25

22. A vaccine composition comprising an effective amount of the polynucleotide of any  
one of claims 7 to 16 and a pharmaceutically effective carrier.



23. The vaccine composition according to either one of claims 21 or 22 wherein said composition comprises at least one other *Neisseria meningitidis* antigen.
24. An antibody immunospecific for the polypeptide or immunological fragment as  
5 claimed in any one of claims 1 to 6.
25. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 - 6, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of  
10 having such an infection.
26. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 – 6 in the preparation of a medicament for use in generating an immune response in an animal.  
15
27. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 7 - 16 in the preparation of a medicament for use in generating an immune response in an animal.
- 20 28. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 – 6 and a suitable pharmaceutical carrier.





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (30) Priority Data:  |             | 9811260.0              | 26 May 1998 (26.05.98)  | GB          |        |             |     |      |            |      |                  |      |
| (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).  |             |                        |   |             |        |             |     |      |            |      |                  |      |
| (72) Inventor; and   |             |                        |   |             |        |             |     |      |            |      |                  |      |
| (75) Inventor/Applicant (for US only): RUELLE, Jean-Louis [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).   |             |                        |   |             |        |             |     |      |            |      |                  |      |
| (74) Agent: TYRRELL, Arthur, William, Russell; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).  |             |                        |   |             |        |             |     |      |            |      |                  |      |
| (54) Title: NEISSERIA MENINGITIDIS ANTIGENIC POLYPEPTIDES, CORRESPONDING POLYNUCLEOTIDES AND PROTECTIVE ANTIBODIES   |             |                        |   |             |        |             |     |      |            |      |                  |      |
| <p style="text-align: center;"><b>Protective effect of the anti-BASB030 antibodies in the passive protection model</b></p> <p style="text-align: center;"><u>Protection against infection</u></p> <table border="1"> <caption>Data from the bar chart showing Log10 (CFU) for different groups.</caption> <thead> <tr> <th>Groups</th> <th>Log10 (CFU)</th> </tr> </thead> <tbody> <tr> <td>PBS</td> <td>~4.2</td> </tr> <tr> <td>H44/29 mab</td> <td>~3.1</td> </tr> <tr> <td>Anti-BASB030 abs</td> <td>~3.0</td> </tr> </tbody> </table> |             |                        |   |             | Groups | Log10 (CFU) | PBS | ~4.2 | H44/29 mab | ~3.1 | Anti-BASB030 abs | ~3.0 |
| Groups   | Log10 (CFU) |                        |   |             |        |             |     |      |            |      |                  |      |
| PBS  | ~4.2        |                        |   |             |        |             |     |      |            |      |                  |      |
| H44/29 mab   | ~3.1        |                        |   |             |        |             |     |      |            |      |                  |      |
| Anti-BASB030 abs   | ~3.0        |                        |   |             |        |             |     |      |            |      |                  |      |
| (57) Abstract  |             |                        |   |             |        |             |     |      |            |      |                  |      |
| <p>The invention provides <i>Neisseria meningitidis</i> BASB030 polypeptides and polynucleotides encoding BASB030 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are antibodies, diagnostic, prophylactic and therapeutic uses thereof.</p>   |             |                        |   |             |        |             |     |      |            |      |                  |      |

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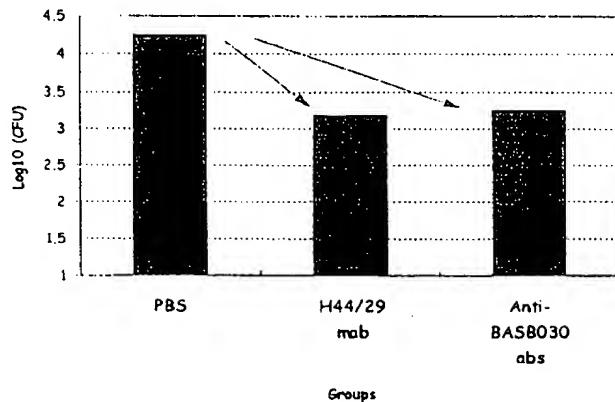
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| C12N 15/31, 1/21, C07K 14/22, 16/12, G01N 33/53, 33/569, A61K 31/70, 39/095, 39/40, 48/00 |   |  |
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| (22) International Filing Date:   | 26 May 1999 (26.05.99)  |  |
| (30) Priority Data:   | 9811260.0 26 May 1998 (26.05.98)  | GB   |
| (71) Applicant (for all designated States except US):                                     | SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).                         |  |
| (72) Inventor; and  |   |  |
| (75) Inventor/Applicant (for US only):  | RUELLE, Jean-Louis [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).     |  |
| (74) Agent:   | TYRRELL, Arthur, William, Russell; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB). |  |
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(54) Title: *NEISSERIA MENINGITIDIS ANTIGENIC POLYPEPTIDES, CORRESPONDING POLYNUCLEOTIDES AND PROTECTIVE ANTIBODIES*

Protective effect of the anti-BASB030 antibodies in the passive protection model

Protection against infection



(57) Abstract

The invention provides *Neisseria meningitidis* BASB030 polypeptides and polynucleotides encoding BASB030 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are antibodies, diagnostic, prophylactic and therapeutic uses thereof.



**NEISSERIA MENINGITIDIS ANTIGENIC POLYPEPTIDES, CORRESPONDING  
POLYNUCLEOTIDES AND PROTECTIVE ANTIBODIES**

**FIELD OF THE INVENTION**

5 This invention relates to polynucleotides, (herein referred to as "BASB030 polynucleotide(s)"), polypeptides encoded by them (referred to herein as "BASB030" or "BASB030 polypeptide(s)"), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including vaccines against bacterial infections. In a further aspect, the  
10 invention relates to diagnostic assays for detecting infection of certain pathogens.

**BACKGROUND OF THE INVENTION**

*Neisseria meningitidis* (meningococcus) is a Gram-negative bacterium frequently isolated from the human upper respiratory tract. It occasionally causes invasive bacterial diseases  
15 such as bacteremia and meningitis. The incidence of meningococcal disease shows geographical seasonal and annual differences (Schwartz, B., Moore, P.S., Broome, C.V.; Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Most disease in temperate countries is due to strains of serogroup B and varies in incidence from 1-10/100,000/year total population sometimes reaching higher values (Kaczmarski, E.B. (1997), Commun. Dis.  
20 Rep. Rev. 1: R55-9, 1995; Scholten, R.J.P.M., Bijlmer, H.A., Poolman, J.T. et al. Clin. Infect. Dis. 16: 237-246, 1993; Cruz, C., Pavez, G., Aguilar, E., et al. Epidemiol. Infect. 105: 119-126, 1990).

Epidemics dominated by serogroup A meningococci, mostly in central Africa, are  
25 encountered, sometimes reaching levels up to 1000/100,000/year (Schwartz, B., Moore, P.S., Broome, C.V. Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Nearly all cases as a whole of meningococcal disease are caused by serogroup A, B, C, W-135 and Y meningococci and a tetravalent A, C, W-135, Y polysaccharide vaccine is available (Armand, J., Arminjon, F., Mynard, M.C., Lafaix, C., J. Biol. Stand. 10: 335-339, 1982).

The polysaccharide vaccines are currently being improved by way of chemical conjugating them to carrier proteins (Lieberman, J.M., Chiu, S.S., Wong, V.K., et al. JAMA 275 : 1499-1503, 1996).

5

A serogroup B vaccine is not available, since the B capsular polysaccharide was found to be nonimmunogenic, most likely because it shares structural similarity to host components (Wyle, F.A., Artenstein, M.S., Brandt, M.L. et al. J. Infect. Dis. 126: 514-522, 1972; Finne, J.M., Leinonen, M., Mäkelä, P.M. Lancet ii.: 355-357, 1983).

10

For many years efforts have been initiated and carried out to develop meningococcal outer membrane based vaccines (de Moraes, J.C., Perkins, B., Camargo, M.C. et al. Lancet 340: 1074-1078, 1992; Bjune, G., Hoiby, E.A. Gronnesby, J.K. et al. 338: 1093-1096, 1991). Such vaccines have demonstrated efficacies from 57% - 85% in older children (>4 years) 15 and adolescents.

15

Many bacterial outer membrane components are present in these vaccines, such as PorA, PorB, Rmp, Opc, Opa, FrpB and the contribution of these components to the observed protection still needs futher definition. Other bacterial outer membrane components have 20 been defined by using animal or human antibodies to be potentially relevant to the induction of protective immunity, such as TbpB and NspA (Martin, D., Cadieux, N., Hamel, J., Brodeux, B.R., J. Exp. Med. 185: 1173-1183, 1997; Lissolo, L., Maître-Wilmotte, C., Dumas, p. et al., Inf. Immun. 63: 884-890, 1995). The mechanisms of protective immunity will involve antibody mediated bactericidal activity and opsonophagocytosis.

25

A bacteremia animal model has been used to combine all antibody mediated mechanisms (Saukkonen, K., Leinonen, M., Abdillahi, H. Poolman, J. T. Vaccine 7: 325-328, 1989). It is generally accepted that the late complement component mediated bactericidal mechanism is

crucial for immunity against meningococcal disease (Ross, S.C., Rosenthal P.J., Berberic, H.M., Densen, P. J. Infect. Dis. 155: 1266-1275, 1987).

The frequency of *Neisseria meningitidis* infections has risen dramatically in the past few  
5 decades. This has been attributed to the emergence of multiply antibiotic resistant strains  
and an increasing population of people with weakened immune systems. It is no longer  
uncommon to isolate *Neisseria meningitidis* strains that are resistant to some or all of the  
standard antibiotics. This phenomenon has created an unmet medical need and demand for  
new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this  
10 organism.

## SUMMARY OF THE INVENTION

The present invention relates to BASB030, in particular BASB030 polypeptides and  
15 BASB030 polynucleotides, recombinant materials and methods for their production. In  
another aspect, the invention relates to methods for using such polypeptides and  
polynucleotides, including prevention and treatment of microbial diseases, amongst others.  
In a further aspect, the invention relates to diagnostic assays for detecting diseases  
associated with microbial infections and conditions associated with such infections, such  
20 as assays for detecting expression or activity of BASB030 polynucleotides or  
polypeptides.

Various changes and modifications within the spirit and scope of the disclosed invention  
will become readily apparent to those skilled in the art from reading the following  
25 descriptions and from reading the other parts of the present disclosure.

## DESCRIPTION OF THE INVENTION

The invention relates to BASB030 polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of BASB030 of *Neisseria meningitidis*, which is related by amino acid sequence homology to *Neisseria gonorrhoeae* PilQ outer membrane protein. The invention relates especially to 5 BASB030 having the nucleotide and amino acid sequences set out in SEQ ID NO:1,3,5 and SEQ ID NO:2,4,6 respectively. It is understood that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of one embodiment of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

10

### Polypeptides

In one aspect of the invention there are provided polypeptides of *Neisseria meningitidis* referred to herein as "BASB030" and "BASB030 polypeptides" as well as biologically, 15 diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% 20 identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2, 4, 6;
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1, 3, 5 over the entire length of SEQ ID NO:1, 3, 5 respectively; or
- 25 (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2, 4, 6;

The BASB030 polypeptides provided in SEQ ID NO:2,4,6 are the BASB030 polypeptides from *Neisseria meningitidis* strains ATCC13090 and H44/76.

The invention also provides an immunogenic fragment of a BASB030 polypeptide, that  
5 is, a contiguous portion of the BASB030 polypeptide which has the same or substantially  
the same immunogenic activity as the polypeptide comprising the amino acid sequence of  
SEQ ID NO:2,4,6. That is to say, the fragment (if necessary when coupled to a carrier) is  
capable of raising an immune response which recognises the BASB030 polypeptide.  
Such an immunogenic fragment may include, for example, the BASB030 polypeptide  
10 lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-  
terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB030  
according to the invention comprises substantially all of the extracellular domain of a  
polypeptide which has at least 85% identity, more preferably at least 90% identity, yet  
more preferably at least 95% identity, most preferably at least 97-99% identity, to that  
15 of SEQ ID NO:2,4,6 over the entire length of SEQ ID NO:2

A fragment is a polypeptide having an amino acid sequence that is entirely the same as part  
but not all of any amino acid sequence of any polypeptide of the invention. As with  
BASB030 polypeptides, fragments may be "free-standing," or comprised within a larger  
20 polypeptide of which they form a part or region, most preferably as a single continuous  
region in a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of an  
amino acid sequence of SEQ ID NO:2,4,6 or of variants thereof, such as a continuous series  
25 of residues that includes an amino- and/or carboxyl-terminal amino acid sequence.  
Degradation forms of the polypeptides of the invention produced by or in a host cell, are  
also preferred. Further preferred are fragments characterized by structural or functional  
attributes such as fragments that comprise alpha-helix and alpha-helix forming regions,  
beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-

forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

- 5 Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO:2,4,6, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO:2,4,6.

10

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these fragments may be employed as intermediates for producing the full-length polypeptides of the invention.

15

Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

- 20 The polypeptides, or immunogenic fragments, of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or  
25 lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various

portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation 5 of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such 10 fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared 15 to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

20 Fusion partners include protein D from *Haemophilus influenzae* and the non-structural protein from influenzae virus, NS1 (hemagglutinin). Another fusion partner is the protein known as LytA. Preferably the C terminal portion of the molecule is used. LytA is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L- 25 alanine amidase, amidase LytA, (coded by the lytA gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LytA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E.coli* C-LytA expressing plasmids useful for

expression of fusion proteins. Purification of hybrid proteins containing the C-LytA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the LytA molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

5

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

10

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced 15 polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

15

It is most preferred that a polypeptide of the invention is derived from *Neisseria meningitidis*, however, it may preferably be obtained from other organisms of the same taxonomic genus. A polypeptide of the invention may also be obtained, for example, from 20 organisms of the same taxonomic family or order.

#### Polynucleotides

25

It is an object of the invention to provide polynucleotides that encode BASB030 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB030.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB030 polypeptides comprising a sequence set out in SEQ ID NO:1,3,5 which includes a full length gene, or a variant thereof.

- 5 The BASB030 polynucleotides provided in SEQ ID NO:1,3,5 are the BASB030 polynucleotides from *Neisseria meningitidis* strains ATCC13090 and H44/76.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB030 polypeptides and polynucleotides, particularly

- 10 *Neisseria meningitidis* BASB030 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

15

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB030 polypeptide having a deduced amino acid sequence of SEQ ID NO:2,4,6 and polynucleotides closely related thereto and variants thereof.

- 20 In another particularly preferred embodiment of the invention there is a BASB030 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:2,4,6 or a variant thereof.

- 25 Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1, 3, 5 a polynucleotide of the invention encoding BASB030 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in

SEQ ID NO:1,3,5, typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:1,3,5 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, each DNA sequence set out in SEQ ID NO:1,3,5 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2, 4, 6 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:1, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 2308 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

The polynucleotide of SEQ ID NO:3, between the first codon at nucleotide number 1 and the last codon which begins at nucleotide number 2308 of SEQ ID NO:3, encodes the polypeptide of SEQ ID NO:4.

The polynucleotide of SEQ ID NO:5, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 2308 of SEQ ID NO:5, encodes the polypeptide of SEQ ID NO:6.

- 5 In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:
- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1,3,5 over the entire length of SEQ ID
  - 10 NO:1,3,5 respectively; or
  - (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:2, 4, 6 over the entire length of SEQ ID NO:2, 4, 6 respectively.

15 A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS

20 concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO: 1, 3, 5 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

25 The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO: 1, 3, 5. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one

non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals.

- 5 The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.
- 10
- 15 The nucleotide sequence encoding BASB030 polypeptide of SEQ ID NO:2, 4, 6 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 2307 of SEQ ID NO:1, or the polypeptide encoding sequence contained in nucleotides 1 to 2307 of SEQ ID NO:3, or the polypeptide encoding sequence contained in nucleotides 1 to 2307 of SEQ ID NO:5, respectively. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2, 4, 6.
- 20

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB030 having an amino acid sequence set out in SEQ ID NO:2, 4, 6. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an

integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode 5 variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:2, 4, 6. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB030 10 variants, that have the amino acid sequence of BASB030 polypeptide of SEQ ID NO:2, 4, 6 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of BASB030 polypeptide.

15 Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB030 polypeptide having an amino acid sequence set out in SEQ ID NO:2, 4, 6, and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% 20 identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

25 Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:1, 3, 5.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB030 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:1, 3, 5.

- 5 The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity  
10 between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C.  
15 Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.  
20 The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1, 3, 5 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1, 3, 5 or a fragment thereof; and isolating said polynucleotide sequence.  
25 Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for

RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB030 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB030 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at 5 least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB030 gene may be isolated by screening using a DNA sequence 10 provided in SEQ ID NO:1, 3, 5 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

15 There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA* 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon<sup>TM</sup> technology (Clontech Laboratories Inc.) for example, have significantly simplified the 20 search for longer cDNAs. In the Marathon<sup>TM</sup> technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers 25 designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly

to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

5 The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

10 The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ ID NOS:1 – 6 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

15 The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may 20 facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

25 For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed

such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may  
5 also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

10

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or  
15 more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

15

In accordance with an aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular  
20 genetic immunization.

20

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff *et al.*, *Hum Mol Genet* (1992) 1: 363, Manthorpe *et al.*, *Hum. Gene Ther.* (1983) 4:  
25 419), delivery of DNA complexed with specific protein carriers (Wu *et al.*, *J Biol Chem.* (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda *et al.*, *Science* (1989) 243: 375), particle bombardment (Tang *et al.*,

*Nature* (1992) 356:152, Eisenbraun *et al.*, *DNA Cell Biol* (1993) 12: 791) and *in vivo* infection using cloned retroviral vectors (Seeger *et al.*, *PNAS USA* (1984) 81: 5849).

5      **Vectors, Host Cells, Expression Systems**

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived  
10     from the DNA constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to  
15     expression systems that comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be  
20     genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor  
25     Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomycetes, cyanobacteria, *Bacillus subtilis*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Neisseria meningitidis*; fungal cells, such as cells of a yeast, *Kluyveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila S2* and *Spodoptera Sf9*; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, 5 hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

- 10 The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), 15 alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelan Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella , Shigella, Neisseria, BCG. These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

20

#### Diagnostic, Prognostic, Serotyping and Mutation Assays

- This invention is also related to the use of BASB030 polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of BASB030 polynucleotides and/or polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a 25 diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the BASB030 gene or protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from any of these sources, particularly DNA or RNA, may be used directly for detection or 5 may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a 10 change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled BASB030 polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched 15 duplexes by DNase or RNase digestion, for DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by 20 direct DNA or RNA sequencing. See, for example, Myers *et al.*, *Science*, 230: 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton *et al.*, *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401 (1985).

25 In another embodiment, an array of oligonucleotides probes comprising BASB030 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to

address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see, for example, Chee *et al.*, *Science*, 274: 610 (1996)).

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

- 5       (a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO:1, 3, 5, or a fragment thereof ;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2, 4, 6 or a fragment thereof; or
- 10     (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2, 4, 6.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease,  
15 among others.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, preferable, SEQ ID NO:1, 3, 5, which is associated with a disease or pathogenicity will  
20 provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such  
25 as those described elsewhere herein.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for

example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose, PCR. As an example, PCR primers complementary to a polynucleotide encoding  
5 BASB030 polypeptide can be used to identify and analyze mutations.

The invention further provides primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying  
BASB030 DNA and/or RNA isolated from a sample derived from an individual, such as a  
10 bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual, such that the polynucleotide may then be subject to various techniques for elucidation of the polynucleotide sequence. In this way, mutations in the polynucleotide sequence may be detected and used to diagnose and/or prognose the infection or its stage or course, or to serotype and/or classify the infectious agent.  
15

The invention further provides a process for diagnosing disease, preferably bacterial infections, more preferably infections caused by *Neisseria meningitidis*, comprising determining from a sample derived from an individual, such as a bodily material, an increased level of expression of polynucleotide having a sequence of SEQ ID NO:1, 3, 5.

20 Increased or decreased expression of a BASB030 polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting, spectrometry and other hybridization methods.

25 In addition, a diagnostic assay in accordance with the invention for detecting over-expression of BASB030 polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a BASB030 polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include

radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

The polynucleotides of the invention may be used as components of polynucleotide arrays, preferably high density arrays or grids. These high density arrays are particularly useful for diagnostic and prognostic purposes. For example, a set of spots each comprising a different gene, and further comprising a polynucleotide or polynucleotides of the invention, may be used for probing, such as using hybridization or nucleic acid amplification, using a probe obtained or derived from a bodily sample, to determine the presence of a particular polynucleotide sequence or related sequence in an individual. Such a presence may indicate the presence of a pathogen, particularly *Neisseria meningitidis*, and may be useful in diagnosing and/or prognosing disease or a course of disease. A grid comprising a number of variants of the polynucleotide sequence of SEQ ID NO:1, 3, 5 are preferred. Also preferred is a grid comprising a number of variants of a polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2, 4, 6.

### Antibodies

The polypeptides and polynucleotides of the invention or variants thereof, or cells expressing the same can be used as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides respectively.

In certain preferred embodiments of the invention there are provided antibodies against BASB030 polypeptides or polynucleotides.

Antibodies generated against the polypeptides or polynucleotides of the invention can be obtained by administering the polypeptides and/or polynucleotides of the invention, or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing either or both, to an animal, preferably a nonhuman, using routine protocols. For

preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this invention. Also, transgenic mice, or other organisms or animals, such as other mammals, 10 may be used to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR 15 amplified v-genes of lymphocytes from humans screened for possessing anti-BASB030 or from naive libraries (McCafferty, *et al.*, (1990), *Nature* 348, 552-554; Marks, *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*, (1991) *Nature* 352: 628).  
20 The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or polynucleotides by, for example, affinity chromatography.

Thus, among others, antibodies against BASB030-polypeptide or BASB030-polynucleotide 25 may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complementarity determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones *et al.* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) *Biotechnology* 9, 266-273.

#### Antagonists and Agonists - Assays and Molecules

- 10 Polypeptides and polynucleotides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, e.g., Coligan *et al.*, *Current Protocols in Immunology* 1(2): Chapter 5 (1991).
- 15 The screening methods may simply measure the binding of a candidate compound to the polypeptide or polynucleotide, or to cells or membranes bearing the polypeptide or polynucleotide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method
- 20 may involve competition with a labeled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by
- 25 the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods

may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring BASB030 polypeptide and/or polynucleotide activity in the mixture, and comparing the BASB030 polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and BASB030 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB030 polypeptides or polynucleotides, particularly those compounds that are bacteriostatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB030 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB030 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB030 polypeptide is

reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of BASB030 polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in BASB030  
5 polynucleotide or polypeptide activity, and binding assays known in the art.  
10

Another example of an assay for BASB030 agonists is a competitive assay that combines BASB030 and a potential agonist with BASB030-binding molecules, recombinant BASB030 binding molecules, natural substrates or ligands, or substrate or ligand mimetics,  
15 under appropriate conditions for a competitive inhibition assay. BASB030 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB030 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

20 Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing  
25 BASB030-induced activities, thereby preventing the action or expression of BASB030 polypeptides and/or polynucleotides by excluding BASB030 polypeptides and/or polynucleotides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists

5 include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991);

*OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*,

CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred

potential antagonists include compounds related to and variants of BASB030.

10 In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the

15 hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion

proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such

20 fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be

25 used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial BASB030 proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided BASB030 agonists and antagonists, preferably bacteristatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

In a further aspect, the present invention relates to mimotopes of the polypeptide of the invention. A mimotope is a peptide sequence, sufficiently similar to the native peptide (sequentially or structurally), which is capable of being recognised by antibodies which recognise the native peptide; or is capable of raising antibodies which recognise the native peptide when coupled to a suitable carrier.

Peptide mimotopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus

distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby presenting the peptide in a conformation which most closely resembles that of the peptide as found in the context of the whole native molecule. For example, the peptides  
5 may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide.

- 10 Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the polypeptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves  
15 share significant sequence homology to the native polypeptide.

### Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises  
20 inoculating the individual with BASB030 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Neisseria meningitidis* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention  
25 relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB030 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB030 polynucleotide and/or polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce

antibody and/or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles 5 or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an 10 immunological response, induces an immunological response in such individual to a BASB030 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB030 polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said BASB030 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the 15 invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A BASB030 polypeptide or a fragment thereof may be fused with co-protein or chemical 20 moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta- 25 galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 5 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial cell surface proteins, in polynucleotide constructs used in such genetic

10 immunization experiments in animal models of infection with *Neisseria meningitidis*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection, 15 for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Neisseria meningitidis* infection, in mammals, particularly humans.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant polypeptide and/or polynucleotide of the invention together with a suitable 20 carrier, such as a pharmaceutically acceptable carrier. Since the polypeptides and polynucleotides may be broken down in the stomach, each is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, 25 buffers, bacteriostatic compounds and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed

ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

5 The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

10 An immune response may be broadly distinguished into two extreme categories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

15 Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

20 It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

25 The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of

cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with

5 the production of the INF- $\gamma$  and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2-type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

10 It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio  
15 of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and  
20 antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

25 3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (European Patent number 0 689 454).

3D-MPL will be present in the range of 10 $\mu$ g - 100 $\mu$ g preferably 25-50 $\mu$ g per dose  
5 wherein the antigen will typically be present in a range 2-50 $\mu$ g per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with a carrier.

10

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have  
15 been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen.

15

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as  
20 disclosed in WO 96/02555.

20

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with  
25 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

- 5 A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

10

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1 $\mu$ g - 200 $\mu$ g, such as 10-100 $\mu$ g, preferably 10 $\mu$ g - 50 $\mu$ g per dose.

Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha

- 15 tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

- 20 Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

25

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a

polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

While the invention has been described with reference to certain BASB030 polypeptides and polynucleotides, it is to be understood that this covers fragments of the naturally occurring polypeptides and polynucleotides, and similar polypeptides and polynucleotides with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

- 10 The antigen can also be delivered in the form of whole bacteria (dead or alive) or as subcellular fractions, these possibilities do include *N.meningitidis* itself.

#### Compositions, kits and administration

- 15 In a further aspect of the invention there are provided compositions comprising a BASB030 polynucleotide and/or a BASB030 polypeptide for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising a polynucleotide and/or a polypeptide discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of  
20 the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are  
25 not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

- 5 The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as  
10 an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such  
15 as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one  
20 or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or  
25 an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present

invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, solutions, powders and the like.

- 5     For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course,  
10    be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the  
15    attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject.

- A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5  
20    microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.
- 25    Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted

using standard empirical routines for optimization, as is well understood in the art.

**Sequence Databases, Sequences in a Tangible Medium, and Algorithms**

5 Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such  
10 as the GCG program package.

Also provided by the invention are methods for the analysis of character sequences or strings, particularly genetic sequences or encoded protein sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such  
15 as identity and similarity analysis, DNA, RNA and protein structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, codon usage analysis, nucleic acid base trimming, and sequencing chromatogram peak analysis.

20 A computer based method is provided for performing homology identification. This method comprises the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and comparing said first polynucleotide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

25 A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a first polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and

comparing said first polypeptide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

10

## DEFINITIONS

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heine, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. 20 *Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GAP 25 program in the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1):

387 (1984)), BLASTP, BLASTN (Altschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990), and FASTA( Pearson and Lipman Proc. Natl. Acad. Sci. USA 85; 2444-2448 (1988). The BLAST family of programs is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; 5 Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

10 Comparison matrix: BLOSUM62 from Henikoff and Henikoff,  
Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 8

Gap Length Penalty: 2

A program useful with these parameters is publicly available as the "gap" program from  
15 Genetics Computer Group, Madison WI. The aforementioned parameters are the default  
parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

20 Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These  
are the default parameters for nucleic acid comparisons.

25

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may  
be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

15

$$n_n \leq x_n - (x_n \circ y),$$

wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\circ$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is it may be 100% identical, or it may include up to a certain integer number of nucleic acid alterations as compared to the

reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of nucleic acids in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleic acids in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \circ y),$$

wherein  $n_n$  is the number of nucleic acid alterations,  $x_n$  is the total number of nucleic acids in SEQ ID NO:1,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc.,  $\circ$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ .

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of

amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

5       $n_a \leq x_a - (x_a \circ y),$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\circ$  is the symbol for 10 the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

By way of example, a polypeptide sequence of the present invention may be identical to 15 the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the 20 reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and 25 then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \circ y),$$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc.. and  $\diamond$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

5

"Individual(s)," when used herein with reference to an organism, means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

10 "Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or  
15 polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

20 "Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions.

25 "Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical

variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including , for example, upper respiratory tract infection, invasive bacterial diseases, such as bacteremia and meningitis.

**EXAMPLES:**

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples  
5 are illustrative, but do not limit the invention.

**Example1: Discovery and confirmatory DNA sequencing of the BASB030 gene from two *N.meningitidis* strains.****10 A: BASB030 in *N. meningitidis* serogroup B strain ATCC13090.**

The BASB030 gene of SEQ ID NO:1 was first discovered in the Incyte PathoSeq database containing unfinished genomic DNA sequences of the *N. meningitidis* strain ATCC13090. The translation of the BASB030 polynucleotide sequence, showed in SEQ ID NO:2, showed significant similarity ( 81 % identity in a 758 amino acids overlap) to the *Neisseria gonorrhoeae* PilQ outer membrane protein.  
15

The sequence of the BASB030 gene was further confirmed experimentally. For this purpose, genomic DNA was extracted from  $10^{10}$  cells of the *N.meningitidis* cells (strain ATCC 13090) using the QIAGEN genomic DNA extraction kit (Qiagen GmbH), and 1 $\mu$ g of this material was submitted to Polymerase Chain Reaction DNA amplification  
20 using primers PilQ1 (5'- GGG G GCTAGC AA TAC CAA ACT GAC AAA AAT CAT TTC C -3') [SEQ ID NO:7] containing an internal *Nhe*I site (underlined) and PilQ2 (5'-GGG G AAGCTT AT AGC GCA GGC TGT TGC CGG C -3') [SEQ ID NO:8] containing an internal *Hind*III site (underlined). This PCR product was gel-purified and subjected to DNA sequencing using the Big Dye Cycle Sequencing kit  
25 (Perkin-Elmer) and an ABI 373A/PRISM DNA sequencer. DNA sequencing was performed on both strands with a redundancy of 2 and the full length sequence was assembled using the SeqMan program from the DNASTAR Lasergene software package. The resulting DNA sequence and deduced polypeptide sequence are shown as SEQ ID NO:3 and SEQ ID NO:4 respectively.

**B: BASB030 in *N. meningitidis* serogroup B strain H44/76.**

The sequence of the BASB030 gene was also determined in another *N. meningitidis* serogroup B strain, the strain H44/76. For this purpose, genomic DNA was extracted  
5 from the *N. meningitidis* strain H44/76 using the experimental conditions presented in Example 1. This material (1 µg) was then submitted to Polymerase Chain Reaction DNA amplification using primers PilQ1 and PilQ2 specific for the BASB030 gene. A ~2300bp DNA fragment was obtained, digested by the *NheI/HindIII* restriction endonucleases and inserted into the corresponding sites of the pET-24b  
10 cloning/expression vector (Novagen) using standard molecular biology techniques (Molecular Cloning, a Laboratory Manual, Second Edition, Eds: Sambrook, Fritsch & Maniatis, Cold Spring Harbor press 1989). Recombinant pET-24b/BASB030 was then submitted to DNA sequencing using the Big Dyes kit (Applied biosystems) and analyzed on a ABI 373/A DNA sequencer in the conditions described by the supplier.  
15 As a result, the polynucleotide and deduced polypeptide sequences, referred to as SEQ ID NO:5 and SEQ ID NO:6 respectively, were obtained. Using the MegAlign program from the DNASTAR package, an alignment of the polynucleotide sequences of SEQ ID NO:1, 3 and 5 was performed, and is displayed in Figure 1; a pairwise comparison of identities is summarized in Table 1, showing that the three BASB030 polynucleotide  
20 gene sequences are all similar at identity level greater than 98.0 %. Using the same MegAlign program, an alignment of the polypeptide sequences of SEQ ID NO:2, 4 and 6 was performed, and is displayed in Figure 2; a pairwise comparison of identities is summarized in Table 2, showing that the three BASB030 protein sequences are all similar at a identity level greater than 95.0 %.  
25 Taken together, these data indicate strong sequence conservation of the BASB030 gene among the two *N. meningitidis* serogroup B strains.

**Table 1: Pairwise identities of the BASB030 polynucleotide sequences ( in % )**

|            | SeqID No:3 | SeqID No:5 |
|------------|------------|------------|
| SeqID No:1 | 99.9       | 98.9       |
| SeqID No:3 |            | 99.0       |

5 Table 2: Pairwise identities of the BASB030 polypeptide sequences ( in % )

|            | SeqID No:4 | SeqID No:6 |
|------------|------------|------------|
| SeqID No:2 | 97.4       | 96.9       |
| SeqID No:4 |            | 99.3       |

**Example 2: Expression and purification of recombinant BASB030 protein in *Escherichia coli*.**

10

The construction of the pET-24b/BASB030 cloning/expression vector was described in Example 1B. This vector harbours the BASB030 gene isolated from the strain H44/76 in fusion with a stretch of 6 Histidine residues, placed under the control of the strong bacteriophage T7 gene 10 promoter. For expression study, this vector was introduced into the *Escherichia coli* strain Novablue (DE3) (Novagen), in which, the gene for the T7 polymerase is placed under the control of the isopropyl-beta-D thiogalactoside (IPTG)-regulatable *lac* promoter. Liquid cultures (100 ml) of the Novablue (DE3) [pET-24b/BASB030] *E. coli* recombinant strain were grown at 37°C under agitation until the optical density at 600nm (OD600) reached 0.6. At that time-point, IPTG was added at a final concentration of 1mM and the culture was grown for 4 additional hours. The culture

was then centrifuged at 10,000 rpm and the pellet was frozen at -20°C for at least 10 hours. After thawing, the pellet was resuspended during 30 min at 25°C in buffer A (6M guanidine hydrochloride, 0.1M NaH<sub>2</sub>PO<sub>4</sub>, 0.01M Tris, pH 8.0), passed three-times through a needle and clarified by centrifugation (20000rpm, 15 min). The sample was  
5 then loaded at a flow-rate of 1ml/min on a Ni<sup>2+</sup> -loaded Hitrap column (Pharmacia Biotech). After passage of the flowthrough, the column was washed successively with 40ml of buffer B (8M Urea, 0.1MNaH<sub>2</sub>PO<sub>4</sub>, 0.01M Tris, pH 8.0), 40ml of buffer C (8M Urea, 0.1MNaH<sub>2</sub>PO<sub>4</sub>, 0.01M Tris, pH 6.3). The recombinant protein BASB030/His6 was then eluted from the column with 30ml of buffer D (8M Urea, 0.1MNaH<sub>2</sub>PO<sub>4</sub>, 0.01M  
10 Tris, pH 6.3) containing 500mM of imidazole and 3ml-size fractions were collected. As shown in Figure 3, a highly enriched (Purity estimated to more than 90% pure in coomassie staining) BASB030/His6 protein, migrating at 85kDa (estimated relative molecular mass), was eluted from the column. This polypeptide was reactive against a mouse monoclonal antibody raised against the 5-histidine motif (see figure 3, lane2).  
15 Moreover, the denatured, recombinant PilQ-His6 protein could be solubilized in a solution devoid of urea. For this purpose, denatured PilQ-His6 contained in 8M urea was extensively dialyzed (2 hours) against buffer R (NaCl 150mM, 10mM NaH<sub>2</sub>PO<sub>4</sub>, Arginine 0.5M pH6.8) containing successively 6M, 4M, 2M and no urea. The corresponding preparation of PilQ remains soluble even after freezing and thawing. Taken  
20 together, these data indicate that the BASB030 gene can be expressed and purified under either a soluble or insoluble, recombinant form (BASB030/His6) in *E.coli*.

Example 3 : Immunization of mice with BASB030 polypeptides and recognition of  
25 the antibody response on recombinant BASB030 polypeptide by Elisa.

Partially purified native BASB030 has been injected three times in BALB/C mice on days 0, 14 and 28 (5 animals/group). This native BASB030 polypeptide was derived directly from a *Neisseria meningitidis* B strain (obtained from J Tommassen). Animals

were injected by the subcutaneous route with 5 $\mu$ g (first injection) and 2  $\mu$ g (second and third injections) of BASB030 polypeptide formulated in SBAS2 (SB62 emulsion containing 5 $\mu$ g MPL and 5 $\mu$ g QS21 per dose) or after adsorption onto AlPO4 (with 5  $\mu$ g MPL). A negative control group consisting of mice immunized with the SBAS2 formulation only (without BASB030 polypeptide) has also been added in the experiment. Mice were bled on days 28 (14 days Post II) and 35 (7 days Post III) in order to detect specific anti-BASB030 antibodies. Specific anti-BASB030 antibodies were measured by Elisa using partially purified recombinant BASB030 polypeptide as coated protein on microplates. Analyses were done on pooled sera (from 5 mice) on 10 Post II only (day 28).

*Recognition of BASB030 epitopes on the recombinant protein, by ELISA*

Briefly, microtiter plates (Maxisorp, Nunc) are coated with 100  $\mu$ l of the recombinant BASB030 solution at around 0.5  $\mu$ g/ml in PBS 2 hours at 37°C. 15 Afterwards, plates are washed three times with 300  $\mu$ l of 150 mM NaCl - 0.05 % Tween 20. Afterwards, they are over-coated with 100  $\mu$ l of PBS-0.3 % casein and incubated for 30 min at room temperature with shaking. Plates are washed again using the same procedure before incubation with antibodies. Animal sera are serially two-fold diluted in PBS-0.3 % casein 0.05 % Tween 20 and put into the microplates (12 dilutions 20 starting at the 1/100 dilution) before incubation at room temperature for 30 min with shaking, before the next identical washing step. Anti-mouse Ig (from rabbit, Dakopatts E0413) conjugated to biotin is used at 1/2000 in PBS-0.3 % casein - 0.05 % Tween 20 to detect mouse anti-BASB030 antibodies. After the last washing step (as before), plates are incubated with a streptavidin-peroxidase complex solution diluted at 1/4000 25 in the same solvant solution for 30 min at room temperature under shaking conditions.

Results illustrated hereafter show that BASB030 polypeptide is highly immunogenic in BALB/C mice, both in SBAS2 emulsion and after adsorption onto AlPO4/MPL (Figure 4). These antibodies, induced after two injections only of the native protein, are able to recognize the recombinant BASB030 polypeptide. The figure shows also that

SBAS2 emulsion is a little more immunogenic than the AlPO4/MPL formulation. The purified recombinant BASB030 polypeptide has also been injected in BALB/C mice for evaluation of its immunogenicity.

5

*Recognition of BASB030 native epitopes on the cells, by whole cell ELISA*

The homologous H44/76 MenB strain (B:15:P1.7, 16) has been used as coated bacteria to detect specific anti-BASB030 antibodies in animal sera. Briefly, microtiter plates (Maxisorp, Nunc) are coated with 100 µl of a 1/10 dilution (in PBS) with a H44/76 bacteria solution from a 6 hours culture, in which bacteria were killed by 400 µg/ml tetracycline. Plates are incubated at 37°C for at least 16 hours until plates are completely dried. Then, they are washed three times with 300 µl of 150 mM NaCl - 0.05 % Tween 20. Afterwards, plates are overcoated with 100 µl of PBS-0.3 % casein and incubated for 30 min at room temperature with shaking. Plates are washed again using the same procedure before incubation with antibodies. Animal sera are serially two-fold diluted in PBS-0.3 % Casein 0.05 % Tween 20 and put into the microplates (12 dilutions starting at the 1/100 dilution) before incubation at room temperature for 30 min with shaking, before the next identical washing step. Anti-mouse Ig (from rabbit, Dakopatts E0413) conjugated to biotin is used at 1/2000 in PBS - 0.3 % casein - 0.05 % Tween 20 to detect mouse anti-BASB030 antibodies. After the last washing step (as before), plates are incubated with a streptavidin-peroxidase complex solution diluted at 1/4000 in the same solvant solution for 30 min at room temperature under shaking conditions.

As shown in Figure 5 hereafter, we can conclude that there is a specific BASB030 antigen recognition on the homologous H44/76 strain in mice immunized with the purified molecule formulated in the SBAS2 emulsion as well as on AlPO4/MPL adjuvant (pool of 5 mice/group were done). Antibody response is higher with the

SBAS2 formulation as observed on the recombinant BASB030 protein. Mice injected with the adjuvant SBAS2 only do not show a clear positive reaction.

5

**Example 4 : Presence of anti-BASB030 antibodies in sera from human convalescent patients.**

In this test, human convalescent sera were tested by western-blotting for the presence of  
10 specific antibodies, using purified recombinant BASB030 protein as well as native  
BASB030 protein (from J Tommassen, Netherlands).

Briefly, 5 $\mu$ g of purified BASB030 protein (recombinant or native) are put into a SDS-PAGE gradient gel (4-20%, Novex, code n°EC60252) for electrophoretic migration.

Proteins are transferred to nitrocellulose sheet

15 (0.45  $\mu$ m, Bio-rad code n° 162-0114) at 100 volts for 1 hour using a Bio-rad Trans-blot system (code n°170-3930). Afterwards, the filter is blocked with PBS - 0.05% Tween 20 overnight at room temperature, before incubation with the human sera. These sera are diluted 100 times in PBS - 0.05% Tween 20, and incubated on the nitrocellulose sheet for two hours at room temperature with gentle shaking, using a mini-blotter  
20 system (Miniprotean, Bio-rad code n° 170-4017). After three repeated washing steps in PBS - 0.05 % Tween 20 for 5 min., the nitrocellulose sheet is incubated at room temperature for 1 hour under gentle shaking with the appropriate conjugate (biotinylated anti-human Ig antibodies, from sheep, Amersham code n°RPN1003, or biotinylated anti-mouse Ig antibodies, from rabbits, Amersham code RPN1001) diluted at 1/500 in  
25 the same washing buffer. The membrane is washed three times as previously, and incubated for 30 min with agitation using the streptavidin-peroxidase complex (Amersham code n°1051) diluted at 1/1000 in the washing buffer. After the last three repeated washing steps, the revelation occurs during the 10-15 min incubation time in a 50 ml solution containing 30 mg 4-chloro-1-naphtol (Sigma), 10 ml methanol, 40 ml of

PBS, and 30 µl of H<sub>2</sub>O<sub>2</sub>. The staining is stopped while washing the membrane several times in distilled water.

Results illustrated in Figures 6 and 7 (Part A) show that 5 to 7 out of 7 convalescent sera  
5 recognize either the native BASB030 protein at different molecular weights (Figure 6) or  
the recombinant BASB030 protein at around 90 kDa (Figure 7). The two convalescent  
sera which react weakly against the recombinant BASB030 polypeptide are n° 261469  
and n°261979 (Figure 7), while on the native BASB030 polypeptide, n° 262117 and  
261979 don't show any clear reaction (Figure 6). Those which react with the highest  
10 intensity are the same on both proteins (recombinant and native). These reactions could  
reflect importance of this polypeptide as a vaccine candidate. The native BASB030  
protein appears to show at least three different bands, which are probably attributed to the  
BASB030 polypeptide, the highest one being identical on both gels (around 90 kDa).  
Native BASB030 polypeptide, directly isolated from bacteria presents degradation  
15 products, as bands around 45 and 35 kDa are clearly visible. In part B of both western-  
blots, is illustrated the reaction of mice antibodies directed against the homolog protein  
from *Neisseria gonorrhoeae* (J. Tommassen, The Netherland). Results illustrate clearly  
that there is a clear cross-reaction between both homolog BASB030 proteins : on the  
recombinant BASB030 protein, there are two clear bands detected, one being the major  
20 band at 90 kDa as recognized by human convalescent sera, the other being at around 70  
kDa (Figure 7). On the native BASB030 protein, mice antibodies recognize not only the  
two major bands at 90 and 45 kDa as seen with convalescent sera, but also bands which  
could be degradation products (around 75, 70, 55, 40, 35 and 25 kDa, see Figure 6).

25 Example 5 : Efficacy of a BASB030 vaccine : activity of anti-BASB030 antibodies.

*Bactericidal activity of anti-BASB030 antibodies on homologous *Neisseria meningitidis* strain.*

The bactericidal activity of animal sera (on pools) has been tested as previously described (1, 2.) with only slight differences. Briefly, the *Neisseria meningitidis* serogroup B (H44/76 strain) is used to determine the bactericidal activity of animal sera. In U-bottom 96 well microplates (NUNC), 50 µl/well of serial two-fold serum dilutions 5 were incubated with 37.5 µl/well of the log phase meningococcal suspension adjusted to  $2.5 \cdot 10^4$  CFU/ml and incubated for 15 min at 37°C with shaking at 210 rpm (Orbital shaker, Forma Scientific). Then, 12.5 µl of the baby rabbit complement (Pel-freeze Biologicals, US) is added before incubation for one more hour in the same conditions. Afterwards, 10 µl aliquots of the mixture from each well were spot onto Mueller-Hinton 10 agar plates containing 1% Isovitalex and 1% of heat inactivated Horse serum before overnight incubation at 37°C with 5% CO<sub>2</sub>. The day after, colonies are counted for each dilution tested and bactericidal titers determined as the dilution of the serum for 50 % killing, compared with the complement control without serum. By this method, individual colonies can be counted up to 100 CFU per spot. Titers are expressed as the 15 dilution which induce 50 % killing, calculated by regression analysis.

Results illustrated in Table 3 show that anti-BASB030 antibodies have a strong bactericidal effect on the H44/76 homologous strain, as also observed with the anti-PorA monoclonal antibody used as positive control. At the 1/2560 dilution, percentage of killing is still very high (91 %).

20

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5

**Table 3 : Bactericidal effect of anti-BASB030 antibodies**

| <u>Antibodies tested</u>                              | <u>Dilutions tested</u> | <u>Killing obtained (%)</u> |
|---|-------------------------|-----------------------------|
| <b>Anti-PorA monoclonal Ab<br/>(Positive control)</b> | 1/10000                 | 100                         |
| <b>Anti-BASB030 polyclonal antibodies</b>             | 1/40                    | 96                          |
|   | 1/80                    | 68                          |
|   | 1/160                   | 56                          |
|   | 1/320                   | 68                          |
|   | 1/640                   | 56                          |
|   | 1/1280                  | 96                          |
|   | 1/2560                  | 91                          |
| <b>Negative control</b>                               | 1/40                    | 0                           |

10

**Example 6 : Efficacy of the anti-BASB030 antibodies in the passive protection model (infant rats).**

15 Anti-BASB030 antibodies obtained from immunized mice (5/group, two groups) have been evaluated for their protective efficacy in the infant rat protective model. The assay measures the clearance activity of the *Neisseria meningitidis* B strain by the antibodies injected 24 hours before the challenge.

Briefly, 100 µl of a 1/10 dilution of a pool of mice sera (with specific anti-BASB030 antibodies) are injected by the intraperitoneal route (IP) into 7days old infant rats

(Sprague Dawley) 24 hours before challenge with live bacteria (day -1). On day 0, infant rats, randomized and passively immunized with mice sera on day -1, were injected with 10 mg of iron dextran in 100 µl by the IP route, 30 min before challenge by the same IP route with  $10^7$  live bacteria (100 µl) from one or more *Neisseria meningitidis* strain H44/76 (B:15:P1.7,16), previously rat passaged (twice). The *Neisseria meningitidis* strain is grown in liquid TSB medium for approximately 2 hours. Bacteria are then diluted in PBS to obtain a  $1.10^8$  CFU/ml suspension. Seven days old infant rats are used for this assay. Groups are composed of 8 rats which are randomly mixed between litters before immunization by the IP injection of 100 µl of a pooled serum to be tested. Three hours after challenge, 20 µl of blood, obtained by cardiac puncture after anesthesia, are diluted in PBS (1/10, 1/100, 1/1000 and 1/10000) and the several dilutions are plated on Mueller Hinton medium for Colony Forming Unit (CFU) counting. Afterwards (24 hours later), the number of CFU is estimated and compared to the number of CFU/ml of blood in infant rats passively immunized with PBS. The control group consisted in PBS injected rats. Weighted means are calculated for each animal and the mean of each group is compared to each other mean.

Results obtained with anti-BASB030 antibodies (see Figure 8) illustrate that these specific antibodies have a clearance effect on the *Neisseria meningitidis* strain H44/76 strain when compared to the negative control group. There is up to 1  $\log_{10}$  of difference observed in favor of the anti-BASB030 antibodies, compared with non-specific antibodies present in mice injected with PBS only. The protective effect observed with our anti-BASB030 antibodies is equivalent to the one obtained with a well characterized anti-PorA monoclonal antibody (H44/29, anti-P1.16).

25 **Legend to Figure 3**

Substantially pure (more than 80%) BASB030 protein fractions were obtained on a 4-20% gradient polyacrylamide gel (NOVEX) under SDS-PAGE conditions in parallel to a protein molecular weight marker. Gels were either stained with Coomassie Blue R250 or analyzed by western blot using an anti-(His5) monoclonal antibody.

**Deposited materials**

5 A deposit containing a *Neisseria meningitidis* Serogroup B strain has been deposited with the American Type Culture Collection (herein "ATCC") on June 22, 1997 and assigned deposit number 13090. The deposit was described as *Neisseria meningitidis* (Albrecht and Ghon) and is a freeze-dried, 1.5-2.9 kb insert library constructed from *N. meningitidis* isolate. The deposit is described in Int. Bull. Bacteriol. Nomencl. Taxon. 8: 1-15 (1958).

10 The *Neisseria meningitidis* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

15 The deposited strain contains the full length BASB030 gene. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

20 The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

|   |                               |
|---|-------------------------------|
| Applicant's or agent's file reference<br>FB/BM45323 | International application No. |
|---|-------------------------------|

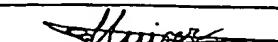
**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

|  |                           |
|--|---------------------------|
| A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>60</u> , line <u>1-22</u>   |                           |
| B. IDENTIFICATION OF DEPOSIT   |                           |
| Name of depositary institution<br><b>AMERICAN TYPE CULTURE COLLECTION</b>  |                           |
| Address of depositary institution ( <i>including postal code and country</i> )<br>10801 UNIVERSITY BLVD, MANASSAS, VIRGINIA 20110-2209,<br>UNITED STATES OF AMERICA  |                           |
| Date of deposit<br>22 June 1997 (22.06.97)   | Accession Number<br>13090 |
| C. ADDITIONAL INDICATIONS ( <i>leave blank if not applicable</i> )   |                           |
| In respect of those designations where a European Patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European Patent or until the date on which the application has been refused or withdrawn, only by issue of such a sample to an expert nominated by the person requesting the sample. |                           |
| D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ( <i>if the indications are not for all designated States</i> )  |                           |
| E. SEPARATE FURNISHING OF INDICATIONS ( <i>leave blank if not applicable</i> )   |                           |
| The indications listed below will be submitted to the International Bureau later ( <i>specify the general nature of the indications e.g., "Accession Number of Deposit"</i> )  |                           |

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This sheet was received with the international application

Authorized officer   
**NATHALIE KUIPER**

For International Bureau use only

This sheet was received by the International Bureau on:

Authorized officer

**CLAIMS:**

1. An isolated polypeptide comprising an amino acid sequence which has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:6.

5           2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:6.

10

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:6.

4. An isolated polypeptide of SEQ ID NO:4 and SEQ ID NO:6.

15

5. An isolated polypeptide of SEQ ID NO:2.

6. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 5 in which the immunogenic activity of said immunogenic fragment is substantially the same  
20       as the polypeptide of SEQ ID NO:4 or SEQ ID NO:6.

7. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 85% identity to the amino acid sequence of SEQ ID NO: 4 or 6 over the entire length of SEQ ID NO: 4 or 6 respectively; or a nucleotide sequence complementary  
25       to said isolated polynucleotide.

8. An isolated polynucleotide comprising a nucleotide sequence that has at least 85% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO: 4 or 6 over the

entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

9. An isolated polynucleotide which comprises a nucleotide sequence which has at least 5 85% identity to that of SEQ ID NO: 3 or 5 over the entire length of SEQ ID NO: 3 or 5 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

10. The isolated polynucleotide as claimed in any one of claims 7 to 9 in which the identity is at least 95% to SEQ ID NO: 3 or 5.

10

11. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:4 or SEQ ID NO:6.

12. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:3 or SEQ 15 ID NO:5.

13. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:4 or SEQ ID NO:6, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID 20 NO:3 or SEQ ID NO:5 or a fragment thereof.

14. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2.

25 15. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1.

16. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, obtainable by screening an appropriate library under stringent

hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof.

17. An expression vector or a recombinant live microorganism comprising an isolated  
5 polynucleotide according to any one of claims 7 - 16.

18. A host cell comprising the expression vector of claim 17 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid sequence that has at least 85% identity to the amino acid sequence selected from the group  
10 consisting of: SEQ ID NO:4 or SEQ ID NO:6.

19. A process for producing a polypeptide comprising an amino acid sequence that has at least 85% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:4 or SEQ ID NO:6 comprising culturing a host cell of claim 18 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.  
15

20. A process for expressing a polynucleotide of any one of claims 7 – 16 comprising transforming a host cell with the expression vector comprising at least one of said  
20 polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

21. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 6 and a pharmaceutically acceptable carrier.  
25

22. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 7 to 16 and a pharmaceutically effective carrier.

23. The vaccine composition according to either one of claims 21 or 22 wherein said composition comprises at least one other *Neisseria meningitidis* antigen.

24. An antibody immunospecific for the polypeptide or immunological fragment as  
5 claimed in any one of claims 1 to 6.

25. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 - 6, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of  
10 having such an infection.

26. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 - 6 in the preparation of a medicament for use in generating an immune response in an animal.

15

27. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 7 - 16 in the preparation of a medicament for use in generating an immune response in an animal.

20

28. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 - 6 and a suitable pharmaceutical carrier.



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**Figure 1 : Alignment of the BASB030 polynucleotide sequences.**  
**Identity to SeqID No:1 is indicated by a dot, and a dash (“-”) indicates a missing nucleotide.**

|          | *       | 20         | *          | 40                   | * |    |
|----------|---------|------------|------------|----------------------|---|----|
| Seqid1 : | ATGAATA | CCAAACTGAC | AAAAATCATT | CCGGTCTTTGTCGCAACCGC | : | 50 |
| Seqid3 : | .....   | .....      | .....      | .....                | : | 50 |
| Seqid5 : | .....   | .....      | .....      | .....                | : | 50 |

60 \* 80 \* 100

Seqid1 : CGCCTTCAGACAGCATCTGCAGGAAACATTACAGACATCAAAGTTCCCT : 100  
Seqid3 : ..... : 100  
Seqid5 : ..... G ..... : 100

\* 120 \* 140 \*  
Seqid1 : CCCTGCCAACAAACAGAAAATCGTCAAAGTCAGCTTGACAAAGAGATT : 150  
Seqid3 : ..... : 150  
Seqid5 : ..... : 150

|          |   |   |     |   |     |       |
|----------|---|---|-----|---|-----|-------|
|          | 160   | * | 180 | * | 200 |       |
| Seqid1 : | GTCAACCCGACC GGCTTCGTAACCTCCTCACCGGCCGCATGCC TTGGAA |   |     |   |     | : 200 |
| Seqid3 : | .....   |   |     |   |     | : 200 |
| Seqid5 : |   |   |     |   |     | : 200 |

\* 220 \* 240 \*  
Seqid1 : CTTTGAACAAACCGGCATTTCCATGGATCAACAGGTACTCGAATATGCCG : 250  
Seqid3 : ..... : 250  
Seqid5 : .....

260 \* 280 \* 300  
Seqid1 : ATCCTCTGTTGAGCAAAATCAGTGCGCACAAAACAGCAGCCGTGCGCGT : 300  
Seqid3 : ..... : 300  
Seqid5 : ..... : 300

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|          |  |   |     |   |
|----------|--|---|-----|---|
| *        | 320  | * | 340 | * |
| Seqid1 : | CTGGTTCTGAATCTGAACAAACCAGGGCCAATACAATACCGAAGTACGC GG | : | 350 |   |
| Seqid3 : | .....  | : | 350 |   |
| Seqid5 : | .....  | : | 350 |   |

|          |  |   |     |   |     |
|----------|--|---|-----|---|-----|
| *        | 360  | * | 380 | * | 400 |
| Seqid1 : | GAACAAAGTTGGATATTCACTAACGAATCGGACGATACCGTGTCCGCC | : | 400 |   |     |
| Seqid3 : | .....  | : | 400 |   |     |
| Seqid5 : | .....  | : | 400 |   |     |

|          |  |   |     |   |
|----------|--|---|-----|---|
| *        | 420  | * | 440 | * |
| Seqid1 : | CCGCACGCCCGCCGTAAAAGCCGCGCCTGCCGCACCGGCAAAACACAG | : | 450 |   |
| Seqid3 : | .....  | : | 450 |   |
| Seqid5 : | .....  | : | 450 |   |

|          |   |   |     |   |     |
|----------|---|---|-----|---|-----|
| *        | 460   | * | 480 | * | 500 |
| Seqid1 : | GGCTGCCGCACCGTCTACCAAGTCCGCAGTATCCGTATCCAAACCC TTTA | : | 500 |   |     |
| Seqid3 : | .-.....   | : | 499 |   |     |
| Seqid5 : | .-.....G.....                                       | : | 499 |   |     |

|          |  |   |     |   |
|----------|--|---|-----|---|
| *        | 520  | * | 540 | * |
| Seqid1 : | CCCCGGCAAAACAACAG-CTGCCGCACCGTTACCGAGTCGTAGTATCC | : | 549 |   |
| Seqid3 : | .....G.....                                      | : | 549 |   |
| Seqid5 : | .....G.....                                      | : | 549 |   |

|          |  |   |     |   |     |
|----------|--|---|-----|---|-----|
| *        | 560  | * | 580 | * | 600 |
| Seqid1 : | GTATCCGCACCGTTCAGCCGGCAAAACACAGAGCGGGGGCATCAGCAA A | : | 599 |   |     |
| Seqid3 : | .....  | : | 599 |   |     |
| Seqid5 : | .....  | : | 599 |   |     |

|          |  |   |     |   |
|----------|--|---|-----|---|
| *        | 620  | * | 640 | * |
| Seqid1 : | ACAACAGACGGCAGCACCAAGCAAAACACAGACGGCAGCACAGCAA AAC | : | 649 |   |
| Seqid3 : | .....  | : | 649 |   |

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Seqid5 : .....G.....G..... : 649

660 \* 680 \* 700  
Seqid1 : AACAGGCAGCACCAGCAAAACAAACCAATATCGATTCCGCAAAGAC : 699  
Seqid3 : ..... : 699  
Seqid5 : ..... : 699

\* 720 \* 740 \*  
Seqid1 : GGCAAAATGCCGGCATTATCGAATTGGCTGCATTGGCTTGCCGGCA : 749  
Seqid3 : ..... : 749  
Seqid5 : ..... : 749

760 \* 780 \* 800  
Seqid1 : GCCCGACATCAGCCAACAGCACGACCACATCGTACGCTGAAAAACC : 799  
Seqid3 : ..... : 799  
Seqid5 : ..... : 799

\* 820 \* 840 \*  
Seqid1 : ATACCCCTGCCGACCACGCTCCAACGCAGTTGGATGTGGCAGACTTAAA : 849  
Seqid3 : ..... : 849  
Seqid5 : ..... : 849

860 \* 880 \* 900  
Seqid1 : ACACCGGTTCAAAAGGTTACGCTGAAACGCCTCAATAACGACACCCAGCT : 899  
Seqid3 : ..... : 899  
Seqid5 : ..... : 899

\* 920 \* 940 \*  
Seqid1 : GATTATCACAAACAGCCGGCAACTGGGAACCTCGTCAACAAATCCGCCGCGC : 949  
Seqid3 : ..... : 949  
Seqid5 : ..... : 949

960 \* 980 \* 1000

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Seqid1 : CCGGATACTTACCTCCAAGTCCTGCCGAAAAACAAAACCTCGAGTCA : 999  
Seqid3 : ..... : 999  
Seqid5 : ..... : 999

\* 1020 \* 1040 \*  
Seqid1 : GGCGCGTGAACAATGCGCCAAACCTCACAGGCCGGAAATCTCCCT : 1049  
Seqid3 : ..... : 1049  
Seqid5 : ..... : 1049

1060 \* 1080 \* 1100  
Seqid1 : TGACTTCCAAGATGTCGAAATCCGCACCATCCTGCAGATTGGCAAAG : 1099  
Seqid3 : ..... : 1099  
Seqid5 : ..... : 1099

\* 1120 \* 1140 \*  
Seqid1 : AATCCGGGATGAACATTGTTGCCAGCGACTCCGTCAACGGCAAATGACC : 1149  
Seqid3 : ..... : 1149  
Seqid5 : .....A..... : 1149

1160 \* 1180 \* 1200  
Seqid1 : CTCTCCCTCAAAGACGTACCTGGGATCAGGCTTGATTGGTTATGCA : 1199  
Seqid3 : ..... : 1199  
Seqid5 : .....G..T..G..... : 1199

\* 1220 \* 1240 \*  
Seqid1 : GGCACGCAACCTCGATATGCGCCAACAAGGGAACATCGTCAACATCGCGC : 1249  
Seqid3 : ..... : 1249  
Seqid5 : ...G.....G.....T..... : 1249

1260 \* 1280 \* 1300  
Seqid1 : CCCGCGACGAGCTGCTTGCCAAAGACAAAGCCTTCTACAGGCCGGAAAAA : 1299  
Seqid3 : ..... : 1299  
Seqid5 : .....C.....A..... : 1299

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\* 1320 \* 1340 \*

Seqid1 : GACATTGCCGATCTAGGCAGCTGTATTCAACAAACTTCCAATTGAAATA : 1349

Seqid3 : ..... : 1349

Seqid5 : .....T.G..T.....C.....G..... : 1349

1360 \* 1380 \* 1400

Seqid1 : CAAAAATGTGGAAGAATTCCGCAGCATCCTGCCTGGACAATGCCGACA : 1399

Seqid3 : ..... : 1399

Seqid5 : ..... : 1399

\* 1420 \* 1440 \*

Seqid1 : CAACCGGAAACCGCAATACGCTTGTCAAGCGGAGGGGCAGCGTGCTGATC : 1449

Seqid3 : ..... : 1449

Seqid5 : .G.....C.....A..... : 1449

1460 \* 1480 \* 1500

Seqid1 : GATCCGCCACCAATACCCTGATTGTTACCGATAACCGCAGCGTCATCGA : 1499

Seqid3 : ..... : 1499

Seqid5 : .....C.....C..... : 1499

\* 1520 \* 1540 \*

Seqid1 : AAAATTCCGAAACTGATTGACGAATTGGACGTACCGCGAACAAAGTGA : 1549

Seqid3 : ..... : 1549

Seqid5 : ..... : 1549

1560 \* 1580 \* 1600

Seqid1 : TGATTGAGGCAGCTATCGTCAAGCGGAGACGGCTTCGCGCGATTTG : 1599

Seqid3 : ..... : 1599

Seqid5 : ..... : 1599

\* 1620 \* 1640 \*

Seqid1 : GGCGTTAAATTGGCGCACAGGCAAGAAAAAGCTGAAAATGATACAAG : 1649

Seqid3 : ..... : 1649

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Seqid5 : ..... : 1649

1660 \* 1680 \* 1700

Seqid1 : CGCATTGGCTGGGGGTAAACTCCGGCTCGGCGGACGATAATGGG : 1699

Seqid3 : ..... : 1699

Seqid5 : ..... : 1699

\* 1720 \* 1740 \*

Seqid1 : GGGCGAAACCAAAATCAACCTGCCGATTACCGCTGCCGAAACAGCATT : 1749

Seqid3 : ..... : 1749

Seqid5 : ..... : 1749

1760 \* 1780 \* 1800

Seqid1 : TCGCTGGTGC CGCGATTCCTCCGGTGCCTGAATTGGAATTGTCCGC : 1799

Seqid3 : ..... : 1799

Seqid5 : ..... : 1799

\* 1820 \* 1840 \*

Seqid1 : ATCCGAATCGCTTTCAAAAACCAAAACGCTTGCCAATCCGCGCGTGCTGA : 1849

Seqid3 : ..... : 1849

Seqid5 : ..... : 1849

1860 \* 1880 \* 1900

Seqid1 : CCCAAAACCGCAAAGAGGCCAAATCGAATCCGGTTACGAAATTCTTT : 1899

Seqid3 : ..... : 1899

Seqid5 : ..... : 1899

\* 1920 \* 1940 \*

Seqid1 : ACCGTAACCTCAATCGCGAACGGCGGCAGCACGACGAACTCAA : 1949

Seqid3 : ..... : 1949

Seqid5 : ..... : 1949

1960 \* 1980 \* 2000

K

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Seqid1 : AAAAGCCGTCTGGGCTGACCGTTACGCCAACATCACGCCGACGGCC : 1999  
Seqid3 : ..... : 1999  
Seqid5 : ..... : 1999

\* 2020 \* 2040 \*  
Seqid1 : AAATCATTATGACCGTAAAATCAACAAGGACTCGCCTGCGCAATGTGCC : 2049  
Seqid3 : ..... : 2049  
Seqid5 : ..... : 2049

2060 \* 2080 \* 2100  
Seqid1 : TCCGGAATCAGACGATCCTGTGTATTCGACCAAAACCTGAATACGCA : 2099  
Seqid3 : ..... : 2099  
Seqid5 : ..... : 2099

\* 2120 \* 2140 \*  
Seqid1 : GGCTATGGTTGAAAACGGCGGCACATTGATTGTCGGCGGTATTTATGAAG : 2149  
Seqid3 : ..... : 2149  
Seqid5 : ..... : 2149

2160 \* 2180 \* 2200  
Seqid1 : AAGACAACGGCAATACGCTGACCAAAGTCCCCCTGTTGGCGACATCCCC : 2199  
Seqid3 : ..... : 2199  
Seqid5 : ..... : 2199

\* 2220 \* 2240 \*  
Seqid1 : GTTATCGGCAACCTCTTAAAACACGCGGGAAAAAAACCGACCGCCGCGA : 2249  
Seqid3 : ..... : 2249  
Seqid5 : ..... : 2249

2260 \* 2280 \* 2300  
Seqid1 : ACTGCTGATTTCATACCCCGAGGATTATGGGTACGGCCGGAACAGCC : 2299  
Seqid3 : ..... : 2299  
Seqid5 : ..... : 2299

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Seqid1 : TGCCTATTGA : 2310  
Seqid3 : ..... : 2310  
Seqid5 : ..... : 2310

526 Rec'd DOD 27 NOV 2000

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**Figure 2 : Alignment of the BASB030 polypeptide sequences.  
Identity to SeqID No:2 is indicated by a dot.**

\* 20 \* 40 \*  
Seqid2 : MNTKLTKIISGLFVATAAFQTASAGNITDIKVSSLVPNQKIVKVSFDKEI : 50  
Seqid4 : ..... : 50  
Seqid6 : ..... : 50

60 \* 80 \* 100 \*  
Seqid2 : VNPTGFVTSSPARIALDFEQTGISMDDQQVLEYADPLLSKISAAQNSSRAR : 100  
Seqid4 : ..... : 100  
Seqid6 : ..... : 100

\* 120 \* 140 \*  
Seqid2 : LVLNLNKPGQYNTTEVRGNKVWIFINESDDTVSAPARPAVKAAPAAPAKQQ : 150  
Seqid4 : ..... : 150  
Seqid6 : ..... : 150

160 \* 180 \* 200 \*  
Seqid2 : GCRTVYQVRSIRIQTLYPGKTTAAPFTESVVSVSAPFSPAKQQAAASAK : 200  
Seqid4 : AAAPSTKSAVSVPKF.T.A.QQ..... : 200  
Seqid6 : AAAPSTKSAVSVPKF.T.A.QQ..... : 200

\* 220 \* 240 \*  
Seqid2 : QQTAAAPAKQQTAAPAKQQAAAPAKQTNIDFRKDGNAGIIELAALGFAGQ : 250  
Seqid4 : ..... : 250  
Seqid6 : ..A.....A..... : 250

260 \* 280 \* 300 \*  
Seqid2 : PDISQQHDHIIIVTLKNHTLPTTLQRSLDVADFKTPVQKVTLKRLNNNDTQL : 300  
Seqid4 : ..... : 300  
Seqid6 : ..... : 300

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\* 320 \* 340 \*  
Seqid2 : IITTAGNWELVNKSAAAPGYFTFQVLPKKQNLESGGVNNAPKTFTGRKISL : 350  
Seqid4 : ..... : 350  
Seqid6 : ..... : 350

360 \* 380 \* 400 \*  
Seqid2 : DFQDVEIRTIQILAKESGMNIVASDSVNGKMTLSLKDVPWDQALDLVMQ : 400  
Seqid4 : ..... : 400  
Seqid6 : ..... : 400

\* 420 \* 440 \*  
Seqid2 : ARNLDMRQQGNIVNIAPRDELLAKDKAFLQAEKDIADLGALYSQNFQLKY : 450  
Seqid4 : ..... : 450  
Seqid6 : .....L..... : 450

460 \* 480 \* 500 \*  
Seqid2 : KNVEEFRSILRLDNADTTGNRNTLVSGRSVLIDPATNTLIVTDTRSVIE : 500  
Seqid4 : ..... : 500  
Seqid6 : .....I..... : 500

\* 520 \* 540 \*  
Seqid2 : KFRKLIDELDVPAQQVMIEARIVEAADGFSRDLGVKFATGKKLKNDTS : 550  
Seqid4 : ..... : 550  
Seqid6 : ..... : 550

560 \* 580 \* 600 \*  
Seqid2 : AFGWGVNSGFGGDDKWGAETKINLPITAAANSISLVRRAISSGALNLESA : 600  
Seqid4 : ..... : 600  
Seqid6 : ..... : 600

\* 620 \* 640 \*  
Seqid2 : SESLSKTKTLANPRVLTQNRKEAKIESGYEIPFTVTSIANGGSNTTELK : 650

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Seqid4 : ..... : 650  
Seqid6 : ..... : 650

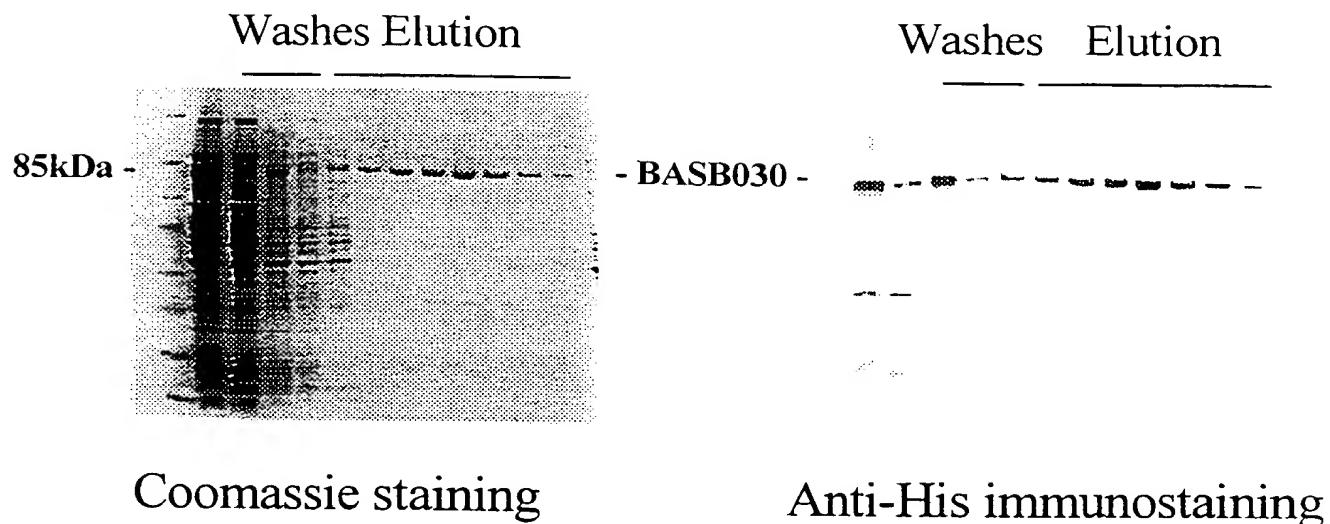
660 \* 680 \* 700  
Seqid2 : KAVLGLTVTPNITPDGQIIMTVKINKDSPAQCASGNQTILCISTKNLNTQ : 700  
Seqid4 : ..... : 700  
Seqid6 : ..... : 700

\* 720 \* 740 \*  
Seqid2 : AMVENGTLIVGGIYEEDNGNTLTKVPLLGDIPVIGNLFKTRGKKDRRE : 750  
Seqid4 : ..... : 750  
Seqid6 : ..... : 750

760  
Seqid2 : LLIFITPRIMGTAGNSLRY : 769  
Seqid4 : ..... : 769  
Seqid6 : ..... : 769

526 Rec'd 11/27 NOV 2000

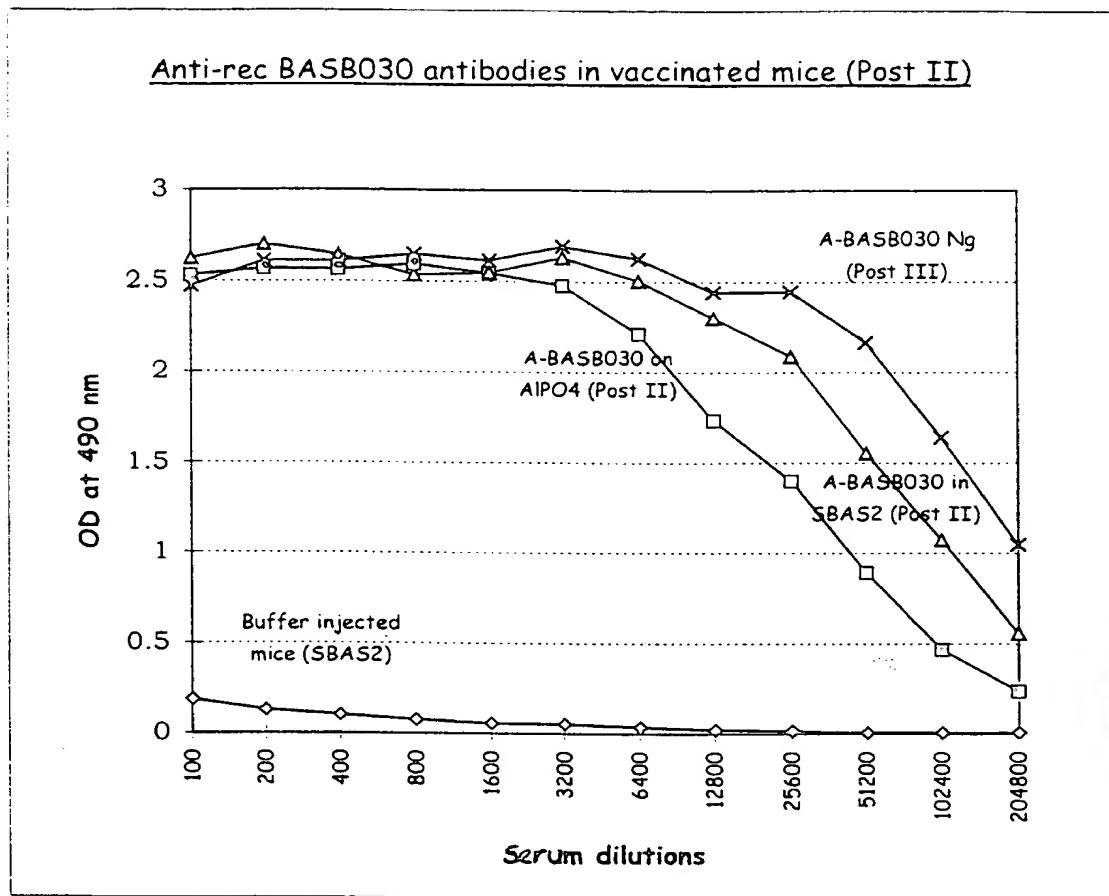
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**Figure 3. Expression and purification of recombinant BASB030 in *E. coli*.**

526 Rec'd FCA/TTO 27 NOV 2000

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Figure 4 : Immunogenicity of the native BASB030 polypeptide. Analysis of the anti-native BASB030 polypeptide on recombinant BASB030 by Elisa.



526 Rec'd NOV 27 2000 27NOV2000

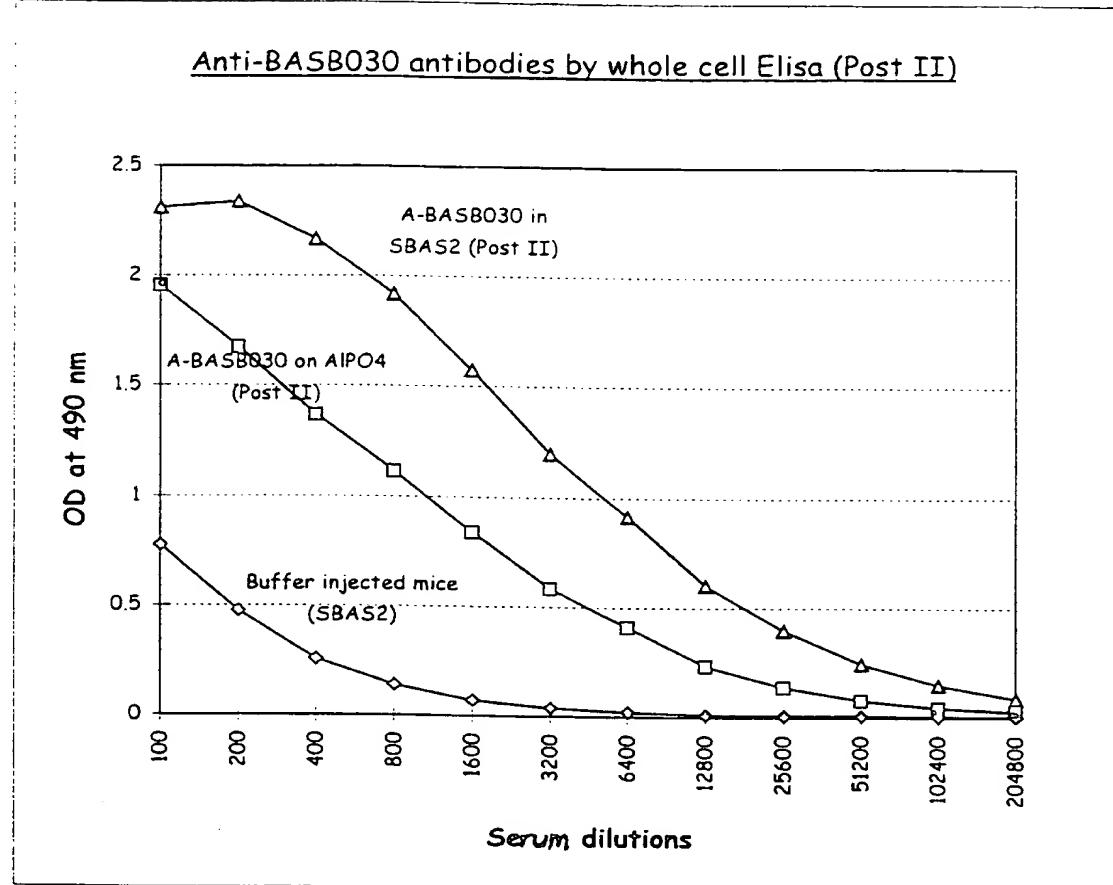
09/701271

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PCT/EP99/03603

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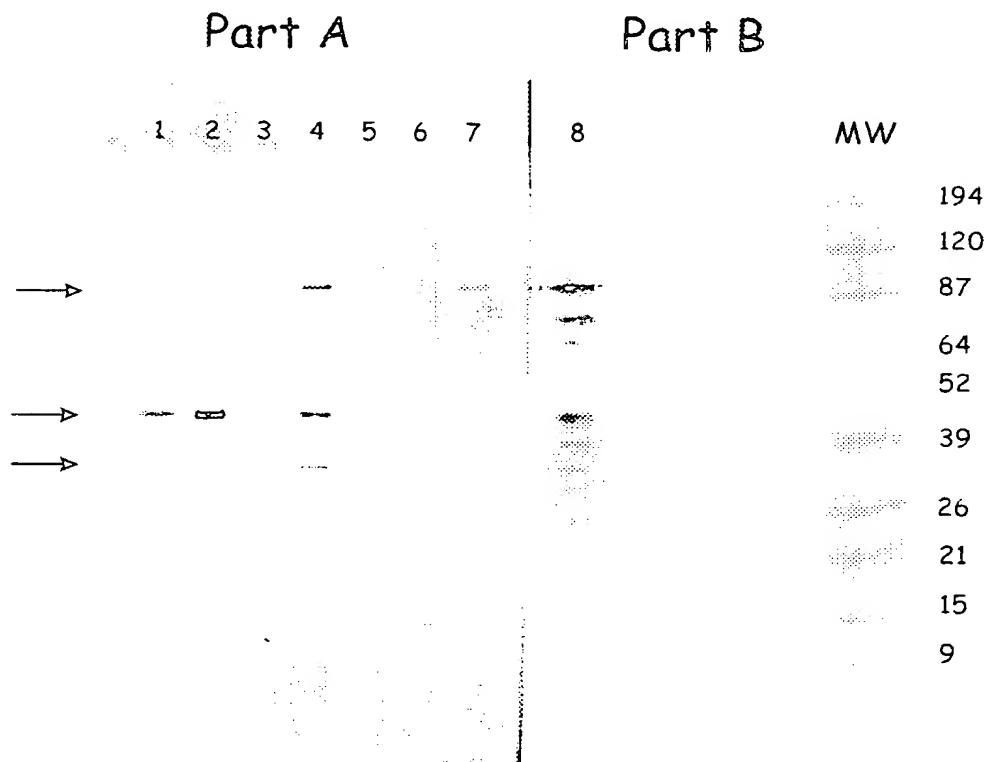
Figure 5 : Immunogenicity of the native BASB030 polypeptide. Analysis of the anti-native BASB030 polypeptide response on whole cells by Elisa.



526 Rec'd FBI/DOJ 27NOV2000

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**Figure 6 : Anti-BASB030 antibodies in human convalescent sera (part A) and in immunized mice (part B) by western-blotting using native BASB030 into the gel.**

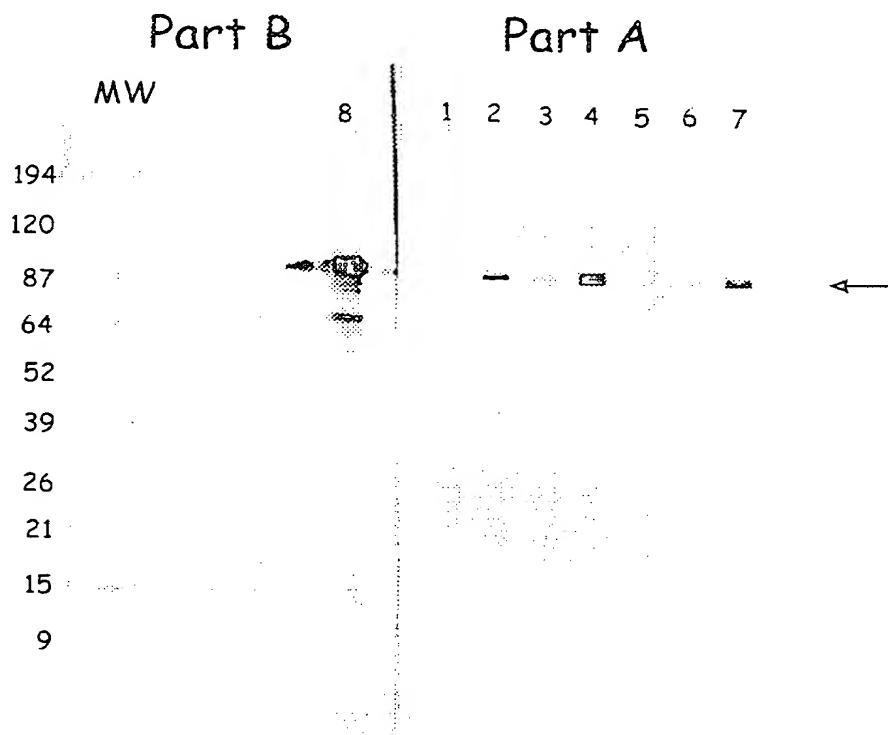


- Lanes :
- 1 : convalescent serum n° 262068
  - 2 : convalescent serum n° 261732
  - 3 : convalescent serum n° 262117
  - 4 : convalescent serum n° 261659
  - 5 : convalescent serum n° 261469
  - 6 : convalescent serum n° 261979
  - 7 : convalescent serum n° 261324
  - 8 : pool of mice sera imunized with the homolog BASB030 protein from Neisseria gonorrhoeae.

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Figure 7 : Anti-BASB030 antibodies in human convalescent sera (part A) and in immunized mice (part B) by western-blotting using recombinant BASB030 protein into the gel.

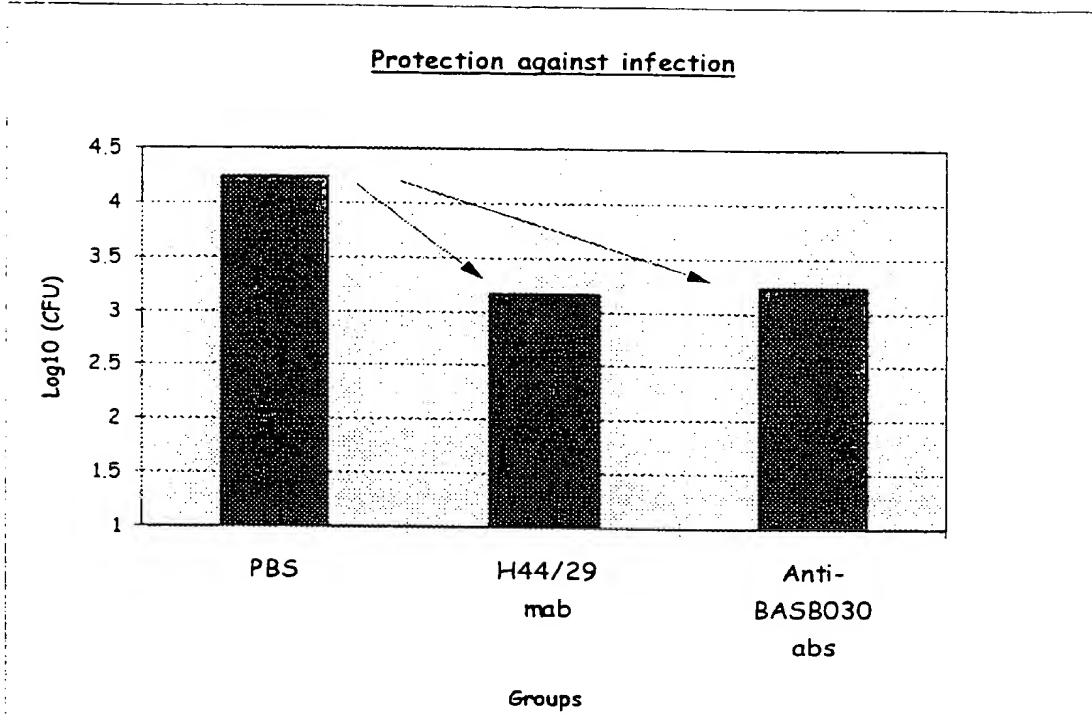


- Lanes :
- 1 : convalescent serum n° 262068
  - 2 : convalescent serum n° 261732
  - 3 : convalescent serum n° 262117
  - 4 : convalescent serum n° 261659
  - 5 : convalescent serum n° 261469
  - 6 : convalescent serum n° 261979
  - 7 : convalescent serum n° 261324
  - 8 : pool of mice sera imunized with the homolog BASB030 protein from Neisseria gonorrhoeae.

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17/17

Figure 8 : Protective effect of the anti-BASB030 antibodies in the passive protection model



526 Rec'd PCT/PTO 27 NOV 2000

## SEQUENCE LISTING

&lt;110&gt; SmithKline Beecham Biologicals S.A.

&lt;120&gt; Novel Compounds

&lt;130&gt; BM45323

&lt;160&gt; 8

&lt;170&gt; FastSEQ for Windows Version 3.0

&lt;210&gt; 1

&lt;211&gt; 2310

&lt;212&gt; DNA

&lt;213&gt; Bacteria

&lt;400&gt; 1

|   |      |
|---|------|
| atgaataccaa aactgacaaa aatcatttcc ggtctctttg tcgcaaccgc cgccttcag   | 60   |
| acagcatctg caggaaacat tacagacatc aaagtttccct ccctgcccaa caaacagaaaa | 120  |
| atcgtaaaag tcagcttga caaagagatt gtcaacccga ccggcttcgt aacctcctca    | 180  |
| ccggcccgca tgcgccttggaa ctggaaacaa accggcattt ccatggatca acaggtactc | 240  |
| gaatatgccc atcctctgtt gagcaaaatc agtgcgcac aaaacagcag ccgtgcgcgt    | 300  |
| ctggttctga atctgaacaa accggccaa tacaataccg aagtacgcgg gaacaaagtt    | 360  |
| tggatattca ttaacgaatc ggacgatacc gtgtccgccc ccgcacgccc cgccgtaaaa   | 420  |
| gccgcgcctg ccgcacccggc aaaacaacag ggctgccgca ccgtctacca agtccgcagt  | 480  |
| atccgtatcc aaacccttta cccccggccaa acaacagctg ccgcaccgtt taccgagtcc  | 540  |
| gtatgtatccg tatccgcacc gttcagcccg gcaaaacaac aggccggccgc atcagcaaaa | 600  |
| caacagacgg cagcaccaggc aaaacaacag acggcagcac cagcaaaaca acaggcggca  | 660  |
| gcaccagcaa aacaacccaa tatcgatttc cgaaagacgc gcaaaaatgc cggcattatc   | 720  |
| gaattggctg cattgggctt tgccggcag cccgacatca gccaaacagca cgaccacatc   | 780  |
| atcgtaacgc tgaaaaacca taccctgccg accacgctcc aacgcagttt ggatgtggca   | 840  |
| gactttaaaa caccggttca aaaggttacg ctgaaacgccc tcaataacga caccagctg   | 900  |
| attatcacaa cagccggcaa ctggaaactc gtcaacaaat ccggccgcgc cggatacttt   | 960  |
| accttccaag tcctgccgaa aaaacaaaac ctcgagtcag gcggcgtgaa caatgcgcgc   | 1020 |
| aaaaccttca caggccggaa aatctccctt gacttccaag atgtcgaaat ccgcaccatc   | 1080 |
| ctgcagattt tggcaaaaga atccggatg aacattgttg ccagcgcactc cgtcaacggc   | 1140 |
| aaaatgaccc tctccctcaa agacgtacct tgggatcagg ctggatgtt ggttatgcag    | 1200 |
| gcacgcacacc tcgatatgcg ccaacaagg aacatcgta acatgcgcgc ccgcgcacgag   | 1260 |
| ctgcttgcca aagacaaagc cttcttacag gcggaaaaag acattgccga tctaggcgcg   | 1320 |



|             |            |             |             |             |            |      |
|-------------|------------|-------------|-------------|-------------|------------|------|
| ctgttattcac | aaaacttcca | attgaaatac  | aaaaatgtgg  | aagaattccg  | cagcatcctg | 1380 |
| cgtttggaca  | atgccgacac | aaccggaaac  | cgcaatacgc  | ttgtcagcgg  | caggggcagc | 1440 |
| gtgttgtatcg | atcccgcac  | caataccctg  | attgttaccg  | atacccgag   | cgtcatcgaa | 1500 |
| aaattccgca  | aactgattga | cgaattggac  | gtacccgcgc  | aacaagtgtat | gattgaggcg | 1560 |
| cgtatcgatcg | aagcggcaga | cggcttctcg  | cgcgattttgg | gcgttaaatt  | cgcgccgaca | 1620 |
| ggcaagaaaa  | agctgaaaaa | tgatacaagc  | gcattcggt   | ggggggtaaa  | ctccggcttc | 1680 |
| ggcggcgcacg | ataaatgggg | ggccgaaaacc | aaaatcaacc  | tgccgattac  | cgctgccgca | 1740 |
| aacagcattt  | cgctggtgcg | cgcgatttcc  | tccggtgct   | tgaatttgg   | attgtccgca | 1800 |
| tccgaatcgc  | tttcaaaaac | caaaccgtt   | gccaatccgc  | gcgtgctgac  | ccaaaaccgc | 1860 |
| aaagaggcca  | aaatcgaatc | cggttacgaa  | attccttca   | ccgtaacctc  | aatcgcaac  | 1920 |
| ggcggcagca  | gcacgaacac | ggaactcaaa  | aaagccgtct  | tggggctgac  | cgttacgccc | 1980 |
| aacatcacgc  | ccgacggcca | aatcattatg  | accgtcaaaa  | tcaacaagga  | ctcgccctg  | 2040 |
| caatgtgcct  | ccggtaatca | gacgatcctg  | tgtatttcg   | ccaaaaacct  | gaatacgcag | 2100 |
| gctatggttg  | aaaacggcgg | cacattgatt  | gtcggcggt   | tttatgaaga  | agacaacggc | 2160 |
| aatacgctga  | ccaaagtccc | cctgttgggc  | gacatcccc   | ttatcgcaa   | cctctttaaa | 2220 |
| acacgcggga  | aaaaaaccga | ccgcccgcgaa | ctgctgattt  | tcattacccc  | gaggattatg | 2280 |
| ggtacggccg  | gcaacagcct | gcgctattga  |             |             |            | 2310 |

&lt;210&gt; 2

&lt;211&gt; 769

&lt;212&gt; PRT

&lt;213&gt; Bacteria

&lt;400&gt; 2

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Asn | Thr | Lys | Leu | Thr | Lys | Ile | Ile | Ser | Gly | Leu | Phe | Val | Ala | Thr |
| 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 15  |
| Ala | Ala | Phe | Gln | Thr | Ala | Ser | Ala | Gly | Asn | Ile | Thr | Asp | Ile | Lys | Val |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 30  |
| Ser | Ser | Leu | Pro | Asn | Lys | Gln | Lys | Ile | Val | Lys | Val | Ser | Phe | Asp | Lys |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 45  |
| Glu | Ile | Val | Asn | Pro | Thr | Gly | Phe | Val | Thr | Ser | Ser | Pro | Ala | Arg | Ile |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 50  |
| Ala | Leu | Asp | Phe | Glu | Gln | Thr | Gly | Ile | Ser | Met | Asp | Gln | Gln | Val | Leu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 65  |
| Glu | Tyr | Ala | Asp | Pro | Leu | Leu | Ser | Lys | Ile | Ser | Ala | Ala | Gln | Asn | Ser |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 85  |
| Ser | Arg | Ala | Arg | Leu | Val | Leu | Asn | Leu | Asn | Lys | Pro | Gly | Gln | Tyr | Asn |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 100 |
| Thr | Glu | Val | Arg | Gly | Asn | Lys | Val | Trp | Ile | Phe | Ile | Asn | Glu | Ser | Asp |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 115 |
| Asp | Thr | Val | Ser | Ala | Pro | Ala | Arg | Pro | Ala | Val | Lys | Ala | Ala | Pro | Ala |



130                    135                    140  
Ala Pro Ala Lys Gln Gln Gly Cys Arg Thr Val Tyr Gln Val Arg Ser  
145                    150                    155                    160  
Ile Arg Ile Gln Thr Leu Tyr Pro Gly Lys Thr Thr Ala Ala Ala Pro  
165                    170                    175  
Phe Thr Glu Ser Val Val Ser Val Ser Ala Pro Phe Ser Pro Ala Lys  
180                    185                    190  
Gln Gln Ala Ala Ala Ser Ala Lys Gln Gln Thr Ala Ala Pro Ala Lys  
195                    200                    205  
Gln Gln Thr Ala Ala Pro Ala Lys Gln Gln Ala Ala Ala Pro Ala Lys  
210                    215                    220  
Gln Thr Asn Ile Asp Phe Arg Lys Asp Gly Lys Asn Ala Gly Ile Ile  
225                    230                    235                    240  
Glu Leu Ala Ala Leu Gly Phe Ala Gly Gln Pro Asp Ile Ser Gln Gln  
245                    250                    255  
His Asp His Ile Ile Val Thr Leu Lys Asn His Thr Leu Pro Thr Thr  
260                    265                    270  
Leu Gln Arg Ser Leu Asp Val Ala Asp Phe Lys Thr Pro Val Gln Lys  
275                    280                    285  
Val Thr Leu Lys Arg Leu Asn Asn Asp Thr Gln Leu Ile Ile Thr Thr  
290                    295                    300  
Ala Gly Asn Trp Glu Leu Val Asn Lys Ser Ala Ala Pro Gly Tyr Phe  
305                    310                    315                    320  
Thr Phe Gln Val Leu Pro Lys Lys Gln Asn Leu Glu Ser Gly Gly Val  
325                    330                    335  
Asn Asn Ala Pro Lys Thr Phe Thr Gly Arg Lys Ile Ser Leu Asp Phe  
340                    345                    350  
Gln Asp Val Glu Ile Arg Thr Ile Leu Gln Ile Leu Ala Lys Glu Ser  
355                    360                    365  
Gly Met Asn Ile Val Ala Ser Asp Ser Val Asn Gly Lys Met Thr Leu  
370                    375                    380  
Ser Leu Lys Asp Val Pro Trp Asp Gln Ala Leu Asp Leu Val Met Gln  
385                    390                    395                    400  
Ala Arg Asn Leu Asp Met Arg Gln Gln Gly Asn Ile Val Asn Ile Ala  
405                    410                    415  
Pro Arg Asp Glu Leu Leu Ala Lys Asp Lys Ala Phe Leu Gln Ala Glu  
420                    425                    430  
Lys Asp Ile Ala Asp Leu Gly Ala Leu Tyr Ser Gln Asn Phe Gln Leu  
435                    440                    445  
Lys Tyr Lys Asn Val Glu Glu Phe Arg Ser Ile Leu Arg Leu Asp Asn  
450                    455                    460



Ala Asp Thr Thr Gly Asn Arg Asn Thr Leu Val Ser Gly Arg Gly Ser  
465 470 475 480  
Val Leu Ile Asp Pro Ala Thr Asn Thr Leu Ile Val Thr Asp Thr Arg  
485 490 495  
Ser Val Ile Glu Lys Phe Arg Lys Leu Ile Asp Glu Leu Asp Val Pro  
500 505 510  
Ala Gln Gln Val Met Ile Glu Ala Arg Ile Val Glu Ala Ala Asp Gly  
515 520 525  
Phe Ser Arg Asp Leu Gly Val Lys Phe Gly Ala Thr Gly Lys Lys Lys  
530 535 540  
Leu Lys Asn Asp Thr Ser Ala Phe Gly Trp Gly Val Asn Ser Gly Phe  
545 550 555 560  
Gly Gly Asp Asp Lys Trp Gly Ala Glu Thr Lys Ile Asn Leu Pro Ile  
565 570 575  
Thr Ala Ala Ala Asn Ser Ile Ser Leu Val Arg Ala Ile Ser Ser Gly  
580 585 590  
Ala Leu Asn Leu Glu Leu Ser Ala Ser Glu Ser Leu Ser Lys Thr Lys  
595 600 605  
Thr Leu Ala Asn Pro Arg Val Leu Thr Gln Asn Arg Lys Glu Ala Lys  
610 615 620  
Ile Glu Ser Gly Tyr Glu Ile Pro Phe Thr Val Thr Ser Ile Ala Asn  
625 630 635 640  
Gly Gly Ser Ser Thr Asn Thr Glu Leu Lys Lys Ala Val Leu Gly Leu  
645 650 655  
Thr Val Thr Pro Asn Ile Thr Pro Asp Gly Gln Ile Ile Met Thr Val  
660 665 670  
Lys Ile Asn Lys Asp Ser Pro Ala Gln Cys Ala Ser Gly Asn Gln Thr  
675 680 685  
Ile Leu Cys Ile Ser Thr Lys Asn Leu Asn Thr Gln Ala Met Val Glu  
690 695 700  
Asn Gly Gly Thr Leu Ile Val Gly Gly Ile Tyr Glu Glu Asp Asn Gly  
705 710 715 720  
Asn Thr Leu Thr Lys Val Pro Leu Leu Gly Asp Ile Pro Val Ile Gly  
725 730 735  
Asn Leu Phe Lys Thr Arg Gly Lys Lys Thr Asp Arg Arg Glu Leu Leu  
740 745 750  
Ile Phe Ile Thr Pro Arg Ile Met Gly Thr Ala Gly Asn Ser Leu Arg  
755 760 765  
Tyr



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 <211> 2310  
 <212> DNA  
 <213> Bacteria

<400> 3

|                                 |                        |                                  |                       |     |
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| acagcatctg cagaaacat tacagacatc | aaagtttcct ccctgcccaa  | caaacagaaaa                      | 120                   |     |
| atcgtaaag ttagcttga caaagagatt  | gtcaacccga ccggcttcgt  | aacctcctca                       | 180                   |     |
| ccggccgc                        | tcgccttgg              | cttgaacaa accggcatt              | ccatggatca acaggtactc | 240 |
| aatatgccg atcctctgtt            | gagaaaatc agtgccgcac   | aaaacagcag ccgtgcgcgt            | 300                   |     |
| ctggttctga atctgaacaa           | accggccaa tacaataccg   | aagtacgcgg gaacaaagtt            | 360                   |     |
| tggatattca ttaacgaatc           | ggacgatacc gttgtccgccc | ccgcacgccc cgccgtaaaa            | 420                   |     |
| gcccgcctg ccgcacccggc           | aaaacaacag gctgcccac   | cgtctaccaa gtccgcagta            | 480                   |     |
| tccgtatcca aaccctttac           | cccgccaaaaa caacaggctg | ccgcacccgtt taccgagtcc           | 540                   |     |
| gtatgtatccg tatccgcacc          | gttcagcccc             | gcaaaaacaac aggccgcggc atcagaaaa | 600                   |     |
| caacagacgg cagcaccaggc          | aaaacaacag acggcagcac  | cagcaaaaca acaggcggca            | 660                   |     |
| gcaccagcaa aacaaaccaa           | tatcgatttc             | cgcaaaagacg gcaaaaatgc           | 720                   |     |
| gaattggctg cattgggctt           | tgccggcag cccgacatca   | gccaacagca                       | 780                   |     |
| atcggtacgc tgaaaaacca           | taccctgccc             | accacgctcc aacgcagttt            | 840                   |     |
| gactttaaaa caccggttca           | aaaggttacg             | ctgaaaacgccc tcaataacga          | 900                   |     |
| attatcacaa cagccggcaa           | ctggaaactc             | cgcaacaaat ccgcgcgc              | 960                   |     |
| accttccaag tcctgccc             | aaaacaaaac             | cgataacttt                       | 1020                  |     |
| aaaaccttca caggccggaa           | aatctccctt             | gacttccaag atgtcgaaat            | 1080                  |     |
| ctgcagat                        | ttggaaaaga             | ccgcaccatc                       | 1140                  |     |
| aaaatgaccc tctccctcaa           | atccggatg              | aacattgttg                       | 1200                  |     |
| gcaacgcgca                      | ccgatcg                | ccagcgactc                       | 1260                  |     |
| ctgcagat                        | ccatccctt              | atgtcgaaat                       | 1320                  |     |
| ctgtattcac aaaacttcca           | attgaaatac             | aaaaatgtgg                       | 1380                  |     |
| cgtttggaca atgcccac             | aaccggaaac             | aaatcgta                         | 1440                  |     |
| gtgctgatcg atcccgcac            | cgcaatacgc             | ttgtcagcgg                       | 1500                  |     |
| aaattccgca aactgattga           | cgaattggac             | ataccgcgc                        | 1560                  |     |
| cgtatcg                         | cgatcg                 | cgatcgatcg                       | 1620                  |     |
| ggcaagaaaa agctgaaaaa           | tgatacaagc             | gcattcggt                        | 1680                  |     |
| ggcggcgcacg                     | ataaaatgggg            | gggggtaaa                        | 1740                  |     |
| aaacagcattt                     | ggccgaaacc             | aaaatcaacc                       | 1800                  |     |
| tccgaatcgc                      | aaaaacgctt             | tgccgattac                       | 1860                  |     |
| aaagaggcca                      | aaatcgatc              | cggttacgaa                       | 1920                  |     |
| ggcggcagca                      | gcacgaacac             | aaagccgtct                       | 1980                  |     |
| aacatcacgc                      | ccgacggcca             | aatcattatg                       | 2040                  |     |
| caatgtgcct                      | ccggtaatca             | gacgatcctg                       | 2100                  |     |



|             |             |            |            |            |            |      |
|-------------|-------------|------------|------------|------------|------------|------|
| gctatggttg  | aaaacggcgg  | cacattgatt | gtcggcgta  | tttatgaaga | agacaacggc | 2160 |
| aatacgtga   | ccaaagtccc  | cctgtgggc  | gacatcccc  | ttatcgcaa  | cctttaaa   | 2220 |
| acacgcggga  | aaaaaaaccga | ccgcccgcga | ctgctgattt | tcattacccc | gaggattatg | 2280 |
| ggtaacggccg | gcaacagcct  | gcgttattga |            |            |            | 2310 |

&lt;210&gt; 4

&lt;211&gt; 769

&lt;212&gt; PRT

&lt;213&gt; Bacteria

&lt;400&gt; 4

|   |                     |     |     |
|---|---------------------|-----|-----|
| Met Asn Thr Lys Leu Thr Lys Ile Ile Ser Gly                     | Leu Phe Val Ala Thr |     |     |
| 1   | 5                   | 10  | 15  |
| Ala Ala Phe Gln Thr Ala Ser Ala Gly Asn Ile Thr Asp Ile Lys Val |                     |     |     |
| 20  | 25                  | 30  |     |
| Ser Ser Leu Pro Asn Lys Gln Lys Ile Val Lys Val Ser Phe Asp Lys |                     |     |     |
| 35  | 40                  | 45  |     |
| Glu Ile Val Asn Pro Thr Gly Phe Val Thr Ser Ser Pro Ala Arg Ile |                     |     |     |
| 50  | 55                  | 60  |     |
| Ala Leu Asp Phe Glu Gln Thr Gly Ile Ser Met Asp Gln Gln Val Leu |                     |     |     |
| 65  | 70                  | 75  | 80  |
| Glu Tyr Ala Asp Pro Leu Leu Ser Lys Ile Ser Ala Ala Gln Asn Ser |                     |     |     |
| 85  | 90                  | 95  |     |
| Ser Arg Ala Arg Leu Val Leu Asn Leu Asn Lys Pro Gly Gln Tyr Asn |                     |     |     |
| 100   | 105                 | 110 |     |
| Thr Glu Val Arg Gly Asn Lys Val Trp Ile Phe Ile Asn Glu Ser Asp |                     |     |     |
| 115   | 120                 | 125 |     |
| Asp Thr Val Ser Ala Pro Ala Arg Pro Ala Val Lys Ala Ala Pro Ala |                     |     |     |
| 130   | 135                 | 140 |     |
| Ala Pro Ala Lys Gln Gln Ala Ala Ala Pro Ser Thr Lys Ser Ala Val |                     |     |     |
| 145   | 150                 | 155 | 160 |
| Ser Val Ser Lys Pro Phe Thr Pro Ala Lys Gln Gln Ala Ala Ala Pro |                     |     |     |
| 165   | 170                 | 175 |     |
| Phe Thr Glu Ser Val Val Ser Val Ser Ala Pro Phe Ser Pro Ala Lys |                     |     |     |
| 180   | 185                 | 190 |     |
| Gln Gln Ala Ala Ala Ser Ala Lys Gln Gln Thr Ala Ala Pro Ala Lys |                     |     |     |
| 195   | 200                 | 205 |     |
| Gln Gln Thr Ala Ala Pro Ala Lys Gln Gln Ala Ala Ala Pro Ala Lys |                     |     |     |
| 210   | 215                 | 220 |     |
| Gln Thr Asn Ile Asp Phe Arg Lys Asp Gly Lys Asn Ala Gly Ile Ile |                     |     |     |
| 225   | 230                 | 235 | 240 |



Glu Leu Ala Ala Leu Gly Phe Ala Gly Gln Pro Asp Ile Ser Gln Gln  
                  245                     250                     255  
 His Asp His Ile Ile Val Thr Leu Lys Asn His Thr Leu Pro Thr Thr  
                  260                     265                     270  
 Leu Gln Arg Ser Leu Asp Val Ala Asp Phe Lys Thr Pro Val Gln Lys  
                  275                     280                     285  
 Val Thr Leu Lys Arg Leu Asn Asn Asp Thr Gln Leu Ile Ile Thr Thr  
                  290                     295                     300  
 Ala Gly Asn Trp Glu Leu Val Asn Lys Ser Ala Ala Pro Gly Tyr Phe  
                  305                     310                     315                     320  
 Thr Phe Gln Val Leu Pro Lys Lys Gln Asn Leu Glu Ser Gly Gly Val  
                  325                     330                     335  
 Asn Asn Ala Pro Lys Thr Phe Thr Gly Arg Lys Ile Ser Leu Asp Phe  
                  340                     345                     350  
 Gln Asp Val Glu Ile Arg Thr Ile Leu Gln Ile Leu Ala Lys Glu Ser  
                  355                     360                     365  
 Gly Met Asn Ile Val Ala Ser Asp Ser Val Asn Gly Lys Met Thr Leu  
                  370                     375                     380  
 Ser Leu Lys Asp Val Pro Trp Asp Gln Ala Leu Asp Leu Val Met Gln  
                  385                     390                     395                     400  
 Ala Arg Asn Leu Asp Met Arg Gln Gln Gly Asn Ile Val Asn Ile Ala  
                  405                     410                     415  
 Pro Arg Asp Glu Leu Leu Ala Lys Asp Lys Ala Phe Leu Gln Ala Glu  
                  420                     425                     430  
 Lys Asp Ile Ala Asp Leu Gly Ala Leu Tyr Ser Gln Asn Phe Gln Leu  
                  435                     440                     445  
 Lys Tyr Lys Asn Val Glu Glu Phe Arg Ser Ile Leu Arg Leu Asp Asn  
                  450                     455                     460  
 Ala Asp Thr Thr Gly Asn Arg Asn Thr Leu Val Ser Gly Arg Gly Ser  
                  465                     470                     475                     480  
 Val Leu Ile Asp Pro Ala Thr Asn Thr Leu Ile Val Thr Asp Thr Arg  
                  485                     490                     495  
 Ser Val Ile Glu Lys Phe Arg Lys Leu Ile Asp Glu Leu Asp Val Pro  
                  500                     505                     510  
 Ala Gln Gln Val Met Ile Glu Ala Arg Ile Val Glu Ala Ala Asp Gly  
                  515                     520                     525  
 Phe Ser Arg Asp Leu Gly Val Lys Phe Gly Ala Thr Gly Lys Lys Lys  
                  530                     535                     540  
 Leu Lys Asn Asp Thr Ser Ala Phe Gly Trp Gly Val Asn Ser Gly Phe  
                  545                     550                     555                     560  
 Gly Gly Asp Asp Lys Trp Gly Ala Glu Thr Lys Ile Asn Leu Pro Ile



|   |     |     |     |
|---|-----|-----|-----|
|   | 565 | 570 | 575 |
| Thr Ala Ala Ala Asn Ser Ile Ser Leu Val Arg Ala Ile Ser Ser Gly |     |     |     |
|   | 580 | 585 | 590 |
| Ala Leu Asn Leu Glu Leu Ser Ala Ser Glu Ser Leu Ser Lys Thr Lys |     |     |     |
|   | 595 | 600 | 605 |
| Thr Leu Ala Asn Pro Arg Val Leu Thr Gln Asn Arg Lys Glu Ala Lys |     |     |     |
|   | 610 | 615 | 620 |
| Ile Glu Ser Gly Tyr Glu Ile Pro Phe Thr Val Thr Ser Ile Ala Asn |     |     |     |
|   | 625 | 630 | 635 |
| Gly Gly Ser Ser Thr Asn Thr Glu Leu Lys Lys Ala Val Leu Gly Leu |     |     |     |
|   | 645 | 650 | 655 |
| Thr Val Thr Pro Asn Ile Thr Pro Asp Gly Gln Ile Ile Met Thr Val |     |     |     |
|   | 660 | 665 | 670 |
| Lys Ile Asn Lys Asp Ser Pro Ala Gln Cys Ala Ser Gly Asn Gln Thr |     |     |     |
|   | 675 | 680 | 685 |
| Ile Leu Cys Ile Ser Thr Lys Asn Leu Asn Thr Gln Ala Met Val Glu |     |     |     |
|   | 690 | 695 | 700 |
| Asn Gly Gly Thr Leu Ile Val Gly Gly Ile Tyr Glu Glu Asp Asn Gly |     |     |     |
|   | 705 | 710 | 715 |
| Asn Thr Leu Thr Lys Val Pro Leu Leu Gly Asp Ile Pro Val Ile Gly |     |     |     |
|   | 725 | 730 | 735 |
| Asn Leu Phe Lys Thr Arg Gly Lys Lys Thr Asp Arg Arg Glu Leu Leu |     |     |     |
|   | 740 | 745 | 750 |
| Ile Phe Ile Thr Pro Arg Ile Met Gly Thr Ala Gly Asn Ser Leu Arg |     |     |     |
|   | 755 | 760 | 765 |
| Tyr   |     |     |     |

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 atcgtcaaag ttagcttga caaagagatt gtcaacccga ccggcttcgt aacctcctca 180  
 ccggccccca tcgccttggc ctttgaacaa accggcattt ccatggatca acaggtactc 240  
 gaatatgccg atcctctgtt gagaaaaatc agtgccgcac aaaacagcag ccgtgcgcgt 300  
 ctggttctga atctgaacaa accggccaa tacaataccg aagtacgcgg gaacaaagtt 360  
 tggatattca ttaacgaatc ggacgatacc gtgtccgccc ccgcacgccc cgccgtaaaa 420



|             |             |             |             |             |            |      |
|-------------|-------------|-------------|-------------|-------------|------------|------|
| gccgcgcctg  | ccgcaccggc  | aaaacaacag  | gctgccgcac  | cgtctaccaa  | gtccgcagta | 480  |
| tccgtatccg  | aacccttac   | ccggcaaaa   | caacaggctg  | ccgcaccgtt  | taccgagtcc | 540  |
| gtagtatccg  | tatccgcacc  | gttcagcccc  | gcaaaaacaac | aggcggcggc  | atcagcaaaa | 600  |
| caacaggcgg  | cagcaccagc  | aaaacaacag  | gccccgacac  | cagcaaaaca  | acaggcggca | 660  |
| gcaccagcaa  | aacaaacccaa | tatcgatttc  | cgcaaagacg  | gcaaaaatgc  | cggcattatc | 720  |
| gaattggctg  | cattgggctt  | tgccggcag   | cccgcacatca | gccaacagca  | cgaccacatc | 780  |
| atcggtacgc  | tgaaaaacca  | taccctgccc  | accacgctcc  | aacgcagttt  | ggatgtggca | 840  |
| gactttaaaa  | caccggttca  | aaagggtacg  | ctgaaacgccc | tcaataacga  | cacccagctg | 900  |
| attatcacaa  | cagccggcaa  | ctggaaactc  | gtcaacaaat  | ccgcccgcgc  | cggatacttt | 960  |
| accttccaag  | tcctgccc    | aaaacaaaac  | ctcgagtcag  | gcggcggtgaa | caatgcgc   | 1020 |
| aaaacccctca | caggccggaa  | aatctccctt  | gacttccaag  | atgtcgaaat  | ccgcaccatc | 1080 |
| ctgcagattt  | tggcaaaaga  | atccggaatg  | aacattgttg  | ccagcgactc  | cgtcaacggc | 1140 |
| aaaatgaccc  | tctccctcaa  | ggatgtgcct  | tgggatcagg  | ctttggattt  | ggttatgcag | 1200 |
| gcgcgc      | caacc       | tcgatatgcg  | ccagcaaggg  | aatatcgtca  | acatgcgc   | 1260 |
| ctgcttgc    | cca         | aagacaaagc  | cctcttacag  | gcagaaaaag  | acattgcga  | 1320 |
| ctgtattccc  | aaaacttcca  | gttgaatac   | aaaaatgtgg  | aagaattccg  | cagcatcctg | 1380 |
| cgttggaca   | atgccc      | gacccggaaac | cgcaacacgc  | ttatcagcgg  | cagggcagc  | 1440 |
| gtgctgatcg  | atcccgcac   | caacaccctg  | attgttaccc  | acacccgcag  | cgtcatcgaa | 1500 |
| aaattccgca  | aactgattga  | cgaattggac  | gtacccgcgc  | aacaagtgtat | gattgaggcg | 1560 |
| cgtatcgtcg  | aagcggcaga  | cggcttc     | cgcgatttgg  | gcgtttaatt  | cggcgcgaca | 1620 |
| ggcaagaaaa  | agctgaaaaa  | tgatacaagc  | gcattcggct  | gggggttaaa  | ctccggcttc | 1680 |
| ggcggc      | gacg        | ataaatgggg  | ggccgaaacc  | aaaatcaacc  | tgccgattac | 1740 |
| aacagcattt  | cgctggcgc   | cgcgatttcc  | tccggcgcct  | tgaatttgg   | attgtccgca | 1800 |
| tccgaatcgc  | tttcaaaaac  | caaaacgctt  | gccaatccgc  | gcgtgctgac  | ccaaaaccgc | 1860 |
| aaagaggcca  | aaatcgaatc  | cggtaacgaa  | attccttca   | ccgtaaacctc | aatcgcgaac | 1920 |
| ggcggc      | gacg        | gcacgaacac  | ggaactcaaa  | aaagccgtct  | tggggctgac | 1980 |
| aacatc      | cacgc       | ccgacggc    | aatcattatg  | accgtcaaa   | tcaacaagga | 2040 |
| caatgtgc    | ctcg        | ccggtaatca  | gacgatcctg  | tgtatcgc    | ccaaaaacct | 2100 |
| gctatggttg  | aaaacggcgg  | cacattgatt  | gtcggcggta  | tttatgaaga  | agacaacggc | 2160 |
| aatacgctga  | ccaaagtccc  | cctgtgggc   | gacatcccc   | ttatcggca   | cctctttaaa | 2220 |
| acacgcggga  | aaaaaaaccga | ccgcgcgaa   | ctgctgattt  | tcattacccc  | gaggattatg | 2280 |
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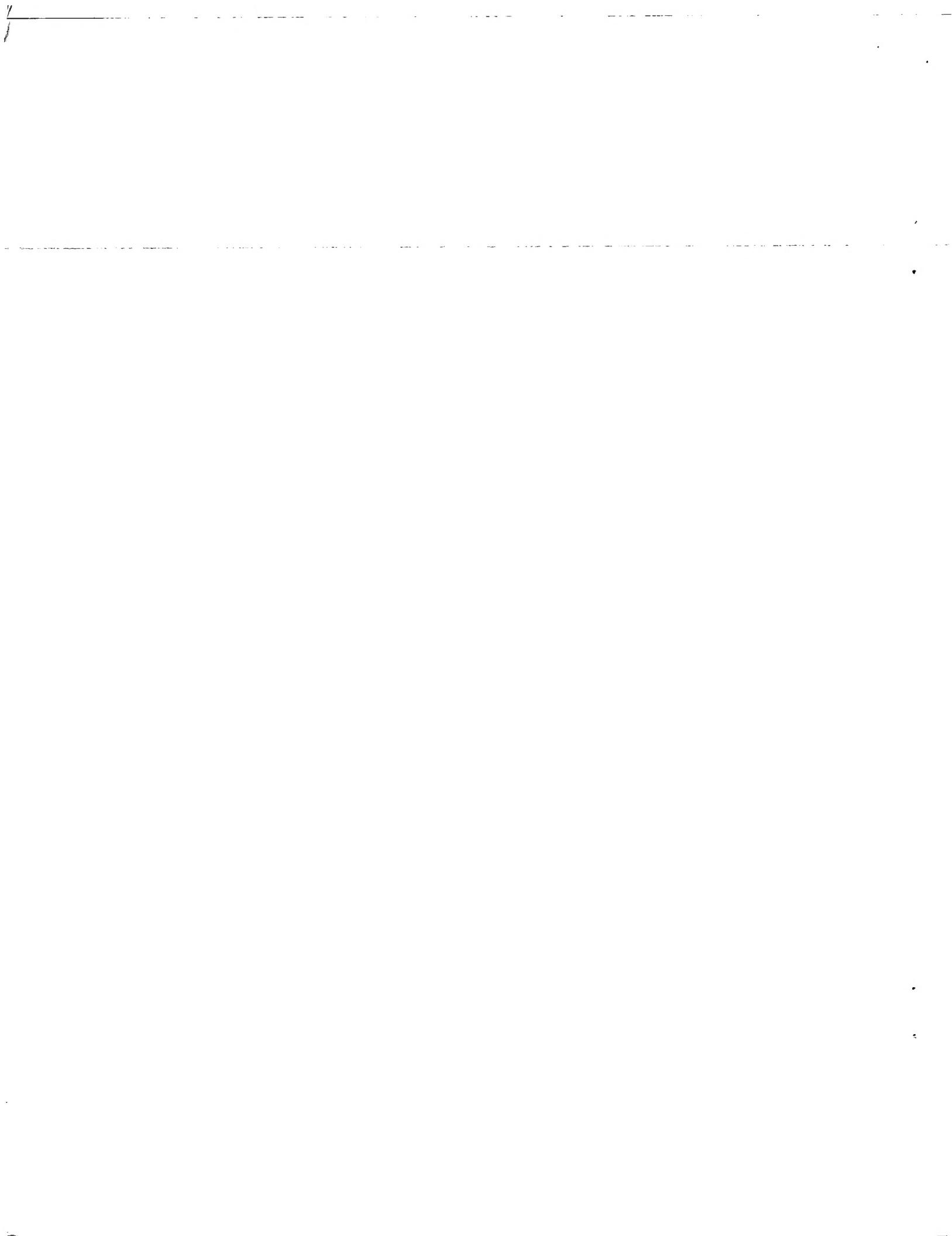
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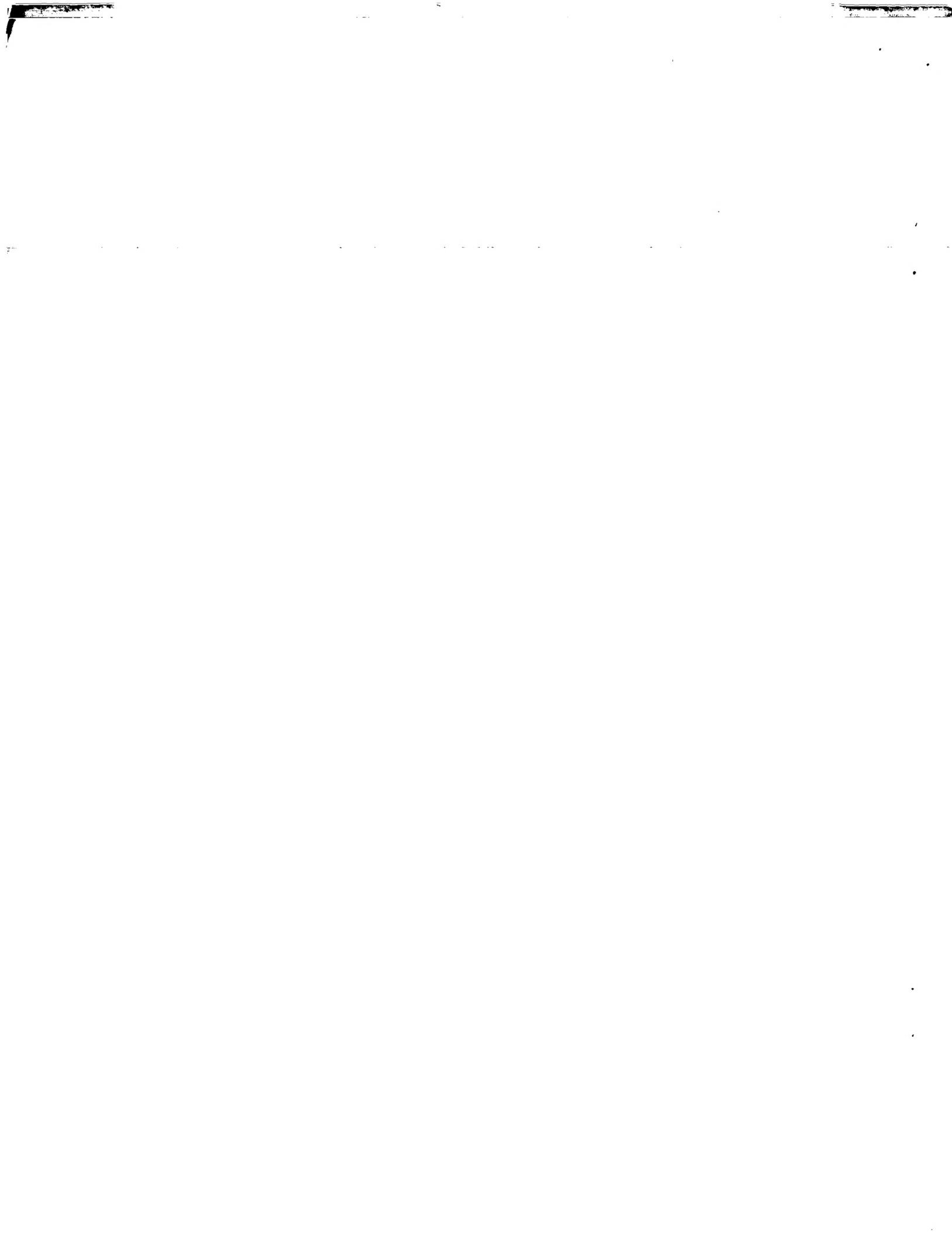
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 Glu Ile Val Asn Pro Thr Gly Phe Val Thr Ser Ser Pro Ala Arg Ile  
           50                     55                     60  
 Ala Leu Asp Phe Glu Gln Thr Gly Ile Ser Met Asp Gln Gln Val Leu  
   65                     70                     75                     80  
 Glu Tyr Ala Asp Pro Leu Leu Ser Lys Ile Ser Ala Ala Gln Asn Ser  
           85                     90                     95  
 Ser Arg Ala Arg Leu Val Leu Asn Leu Asn Lys Pro Gly Gln Tyr Asn  
           100                    105                    110  
 Thr Glu Val Arg Gly Asn Lys Val Trp Ile Phe Ile Asn Glu Ser Asp  
           115                    120                    125  
 Asp Thr Val Ser Ala Pro Ala Arg Pro Ala Val Lys Ala Ala Pro Ala  
           130                    135                    140  
 Ala Pro Ala Lys Gln Gln Ala Ala Ala Pro Ser Thr Lys Ser Ala Val  
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 Ser Val Ser Glu Pro Phe Thr Pro Ala Lys Gln Gln Ala Ala Ala Pro  
           165                    170                    175  
 Phe Thr Glu Ser Val Val Ser Val Ser Ala Pro Phe Ser Pro Ala Lys  
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 Gln Gln Ala Ala Ala Ser Ala Lys Gln Gln Ala Ala Ala Pro Ala Lys  
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 Gln Gln Ala Ala Ala Pro Ala Lys Gln Gln Ala Ala Ala Pro Ala Lys  
           210                    215                    220  
 Gln Thr Asn Ile Asp Phe Arg Lys Asp Gly Lys Asn Ala Gly Ile Ile  
   225                     230                     235                     240  
 Glu Leu Ala Ala Leu Gly Phe Ala Gly Gln Pro Asp Ile Ser Gln Gln  
           245                    250                    255  
 His Asp His Ile Ile Val Thr Leu Lys Asn His Thr Leu Pro Thr Thr  
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 Leu Gln Arg Ser Leu Asp Val Ala Asp Phe Lys Thr Pro Val Gln Lys  
           275                    280                    285  
 Val Thr Leu Lys Arg Leu Asn Asn Asp Thr Gln Leu Ile Ile Thr Thr  
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 Ala Gly Asn Trp Glu Leu Val Asn Lys Ser Ala Ala Pro Gly Tyr Phe  
   305                     310                     315                     320  
 Thr Phe Gln Val Leu Pro Lys Lys Gln Asn Leu Glu Ser Gly Gly Val  
           325                    330                    335  
 Asn Asn Ala Pro Lys Thr Phe Thr Gly Arg Lys Ile Ser Leu Asp Phe



|   |     |     |
|---|-----|-----|
| 340   | 345 | 350 |
| Gln Asp Val Glu Ile Arg Thr Ile Leu Gln Ile Leu Ala Lys Glu Ser |     |     |
| 355   | 360 | 365 |
| Gly Met Asn Ile Val Ala Ser Asp Ser Val Asn Gly Lys Met Thr Leu |     |     |
| 370   | 375 | 380 |
| Ser Leu Lys Asp Val Pro Trp Asp Gln Ala Leu Asp Leu Val Met Gln |     |     |
| 385   | 390 | 395 |
| Ala Arg Asn Leu Asp Met Arg Gln Gln Gly Asn Ile Val Asn Ile Ala |     |     |
| 405   | 410 | 415 |
| Pro Arg Asp Glu Leu Leu Ala Lys Asp Lys Ala Leu Leu Gln Ala Glu |     |     |
| 420   | 425 | 430 |
| Lys Asp Ile Ala Asp Leu Gly Ala Leu Tyr Ser Gln Asn Phe Gln Leu |     |     |
| 435   | 440 | 445 |
| Lys Tyr Lys Asn Val Glu Glu Phe Arg Ser Ile Leu Arg Leu Asp Asn |     |     |
| 450   | 455 | 460 |
| Ala Asp Thr Thr Gly Asn Arg Asn Thr Leu Ile Ser Gly Arg Gly Ser |     |     |
| 465   | 470 | 475 |
| Val Leu Ile Asp Pro Ala Thr Asn Thr Leu Ile Val Thr Asp Thr Arg |     |     |
| 485   | 490 | 495 |
| Ser Val Ile Glu Lys Phe Arg Lys Leu Ile Asp Glu Leu Asp Val Pro |     |     |
| 500   | 505 | 510 |
| Ala Gln Gln Val Met Ile Glu Ala Arg Ile Val Glu Ala Ala Asp Gly |     |     |
| 515   | 520 | 525 |
| Phe Ser Arg Asp Leu Gly Val Lys Phe Gly Ala Thr Gly Lys Lys Lys |     |     |
| 530   | 535 | 540 |
| Leu Lys Asn Asp Thr Ser Ala Phe Gly Trp Gly Val Asn Ser Gly Phe |     |     |
| 545   | 550 | 555 |
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| 565   | 570 | 575 |
| Thr Ala Ala Ala Asn Ser Ile Ser Leu Val Arg Ala Ile Ser Ser Gly |     |     |
| 580   | 585 | 590 |
| Ala Leu Asn Leu Glu Leu Ser Ala Ser Glu Ser Leu Ser Lys Thr Lys |     |     |
| 595   | 600 | 605 |
| Thr Leu Ala Asn Pro Arg Val Leu Thr Gln Asn Arg Lys Glu Ala Lys |     |     |
| 610   | 615 | 620 |
| Ile Glu Ser Gly Tyr Glu Ile Pro Phe Thr Val Thr Ser Ile Ala Asn |     |     |
| 625   | 630 | 635 |
| Gly Gly Ser Ser Thr Asn Thr Glu Leu Lys Lys Ala Val Leu Gly Leu |     |     |
| 645   | 650 | 655 |
| Thr Val Thr Pro Asn Ile Thr Pro Asp Gly Gln Ile Ile Met Thr Val |     |     |
| 660   | 665 | 670 |



Lys Ile Asn Lys Asp Ser Pro Ala Gln Cys Ala Ser Gly Asn Gln Thr  
675 680 685  
Ile Leu Cys Ile Ser Thr Lys Asn Leu Asn Thr Gln Ala Met Val Glu  
690 695 700  
Asn Gly Gly Thr Leu Ile Val Gly Gly Ile Tyr Glu Glu Asp Asn Gly  
705 710 715 720  
Asn Thr Leu Thr Lys Val Pro Leu Leu Gly Asp Ile Pro Val Ile Gly  
725 730 735  
Asn Leu Phe Lys Thr Arg Gly Lys Lys Thr Asp Arg Arg Glu Leu Leu  
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